PB84-155563

Analysis and Assessment of Incinerated Municipal Sludge Ashes and Leachates

Notre Dame Univ., IN

Prepared for

Municipal Environmental Research Lab.
Cincinnati, OH

Feb 84

U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-155563

EPA-600/2-84-038
February 1984

ANALYSIS AND ASSESSMENT OF INCINERATED MUNICIPAL SLUDGE ASHES AND LEACHATES

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Grant R-806-690-01

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CINCINNATI, OHIO 45268

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/2-84-038		PB84 155563

4. TITLE AND SUBTITLE	5. REPORT DATE
ANALYSIS AND ASSESSMENT OF INCINERATED MUNICIPAL SLUDGE ASHES AND LEACHATES	February 1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Thomas L. Theis, Margaret McKiernan and Lawrence E. Padgett	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Notre Dame Notre Dame, IN 46556	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO. R806-690-01
12. SPONSORING AGENCY NAME AND ADDRESS Municipal Environmental Research Laboratory - Cin., OH Office of Research and Development U.S. Environmental Protection Agency Cincinnati, OH 45268	13. TYPE OF REPORT AND PERIOD COVERED Final Oct. 79-Sep 81
	14. SPONSORING AGENCY CODE EPA/600/14

15. SUPPLEMENTARY NOTES
Project Officer: Atal E. Eralp (513)684-2621

16. ABSTRACT Research was conducted to analyze the physical and chemical properties of ashes from incinerated municipal sludge and of corresponding dewatered sludge. Samples were gathered from 10 wastewater treatment plants ranging in size from 0.22 to 27.1 m^3/sec. These samples were subjected to a series of physical, chemical, and biological tests, including batch and chemical extractions and the Ames Salmonella assay.

Results showed that the addition of supplemental chemicals during wastewater or sludge treatment strongly influenced the leachate properties and composition of both dewatered sludge and ash samples. Both iron (III) and aluminum salts acted to concentrate trace metals, which were then readily leached from the sludge ashes. The addition of lime during treatment elevated the ash and sludge pH values, which determine the availability of metals to the environment. In the absence of inorganic chemical addition, incineration acted generally to decrease trace metal availability.

The behavior of arsenic during the various leaching tests was the opposite of that for metal cations with respect to pH. Low pH values resulted in the release of larger numbers of cations, but arsenic release was usually greatest at high pH values.

Incinerated sludges demonstrated weak mutagenic activity in the Ames assay. Four of the 10 ash samples gave positive results, generally in the presence of activating mammalian enzymes. In addition, one dewatered sludge sample also gave positive results. Both frameshift and base-pair mutagens were implicated.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	102
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (9-73)

DISCLAIMER

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution, and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and is a most vital communications link between the researcher and the user community.

The objectives of this study were to physically and chemically characterize several ashes generated from the incineration of municipal wastewter sludges, and to relate these characteristics to the composition and properties of leachates generated under controlled testing conditions.

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ABSTRACT

Incinerated municipal sludge ash and corresponding dewatered sludge samples were gathered from 10 wastewater treatment plants ranging in size from 0.22 m^3/sec to 27.1 m^3/sec. These samples were subjected to a series of physical, chemical, and biological tests, including optical and electron microscopy, batch and serial chemical extractions, and toxicity (mutagenicity) assays.

Results showed that the addition of supplemental chemicals during wastewater or sludge treatment processes strongly influenced the leachate properties and composition of both dewatered sludge and ash samples. Both iron(III) and aluminum salts acted to concentrate trace metals, with the result that they were more readily leached from sludge ashes. The addition of lime during treatment resulted in elevated pH values, pH of the ash or sludge being very important in defining the degree of availability of metals to the environment. In the absence of inorganic chemical addition, incineration acted generally to decrease trace metal availability. Serial extraction tests suggested that this result was due to the greater degree of crystallinity of the major minerals brought about during incineration.

The behavior of arsenic during the various leach tests was opposite that of metal cations with respect to pH. Lower pH values resulted in greater numbers of metal cations being released, but arsenic release was usually greatest at higher pH values.

Ames testing of various extracts of sludge ashes showed that 7 of the 10 samples exhibited some degree of mutagenic activity. The greatest mutagenic response occurred in extraction procedures designed to remove and concentrate refractory organics from the ashes, suggesting that such compounds are responsible for the positive results. Testing of one dewatered sludge also yielded a positive result. It appears appropriate to conduct further testing in order to make comparisons between sludge and the incinerated sludge ash.

This report was submitted in fulfillment of Contract No. R806690 by the Department of Civil Engineering of the University of Notre Dame under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period October 1, 1979 to September 1, 1981, and work was completed as of September 1, 1981.

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SECTION 1

INTRODUCTION

In the United States, slightly more than 4.5 million metric tons (5 million tons) of municipal wastewater sludge solids are produced yearly. This is expected to increase to 6 million metric tons (6.6 million tons) per year by 1985[1,2]. At the present time, approximately 30 percent of these solids are thermally reduced in volume by incineration methods, the most popular of which are the multiple hearth and fluidized bed types[3,4]. Using an average figure of 75 percent for the volatile fraction of municipal sludge gives about 338,000 metric tons (413,000 tons) of ash residue which must be disposed of yearly. The most common disposal methods are landfilling and lagooning, the latter being popular due to the ease of transporting the ash via aqueous slurry pipelines[3,5].

Because of the initial cost and complexity of operation of sludge incinerators, their use is confined mostly to wastewater treatment plants with capacities greater than .044 m^3/sec (1 MGD), with most being greater than 0.22 m^3/sec (5 MGD)[3]. Thus while the total quantities of ash generated are not as great as other types of combustion wastes (e.g. incinerated solid waste, coal fly ash), the disposal can be a sizeable operation on a local basis, especially for larger urban areas.

Conventional wastewater treatment processes can be surprisingly efficient in retaining many objectionable trace inorganic and organic constituents that enter the plant[6,7,8]. Trace organic compounds would be expected to be altered and lost through the biodegradation and incineration processes, however inorganic substances, with few exceptions exhibit more conservative behavior. Successive solids concentration operations, culminating in incineration, often result in mass concentrations of pollutants in excess of what are normally considered trace levels.

Handling of the ash from both of the commonly used incineration methods often involves contact with water. In the fluidized bed system, the ash is removed from the exit stream by a scrubber; for the multiple hearth system ash handling can be either wet (for slurry-pipelines) or dry, however even for dry handling the ash is usually conditioned for disposal with water. Consequently, the behavior of ash in an aqueous environment is of substantial interest. The objectives of this study were to physically and chemi-

cally characterize several sludge ashes, and relate these characteristics to the composition and properties of leachates generated under controlled testing conditions. Particular attention has been focused on the release of trace inorganic species and the mutagenic potential of leachates as defined by the Ames assay.

This report presents a review of available literature on incinerated municipal sludge ash and reports on the present level of understanding of the physical and chemical properties of this material. Particular attention is paid to the studies that have addressed the quantity and extent of the release of trace pollutants into leachate streams. A brief review is also made of the literature on environmental mutagenesis testing. Procedures are discussed for the sampling and testing of ash and dewatered sludge samples conducted during this study. Aspects of both leachate and mutagenic testing are included. Results are discussed, and relevant data trends are identified.

SECTION 2

CONCLUSIONS

1. The addition of $FeCl_3$ and/or alum tends to partition metals from organic to inorganic forms in raw municipal wastewater sludges. This acts, in general, to increase the leachability (as measured by the EPA-EP) of cationic elements while decreasing the leachability of arsenic.

2. Upon incineration the inorganic fractions of Fe and Al become more crystalline. This tends to decrease the leachability of cationic elements in the EPA-EP test when Fe(III)/Al(III) salts are not added during the treatment process. However incineration acts to increase leachability of metal cations when these chemicals are added (except for cadmium).

3. In municipal sludge ashes, the elements Ni, Cr, and As tend to behave as if they were associated with surface oxyhydroxides of iron, whereas Pb and Cd tend to associate with aluminum oxides.

4. The ranking of single-reagent leachate tests according to total amount of trace cationic element removal is (except for Ni)

   EPA-EP > IAEA > ASTM-A

5. Arsenic release in aqueous solution behaves in an inverse fashion to cationic elements; i.e., it is less easily leached in the EPA-EP test when $FeCl_3$/alum have been added during treatment. However the IAEA test consistently removed the greatest total amount of arsenic from sludge ashes.

6. The leaching behavior of all elements is strongly influenced by solution pH.

7. Seven of 10 municipal sludge ashes exhibited weak to moderate mutagenic activity in the Ames Salmonella assay.

8. The mutagenic activity was greatest for aqueous extracts that were concentrated on an XAD type resin.

9. Most positive results occurred in the presence of activating mammalian enzymes.

10. Incineration of sludge does not appear to decrease mutagenic response.

SECTION 3

RECOMMENDATIONS

1. It is recommended that more municipal sludge ashes be tested using procedures similar to those used in this study so that the data trends found can be further verified.

2. It may be advisable to establish guidelines for the handling and disposal of sludge ashes which realistically reflect the nature of the materials and type of disposal environment under consideration.

3. Further studies, perhaps utilizing GC/MS methods, should be done on the organic fraction of sludge ashes in order to identify those compounds responsible for the positive response in the Ames assay.

4. Specific attempts at modeling sorption phenomenon at heterogeneous ash and sludge particle surfaces would be a valuable contribution to the understanding of which reactions are dominant in aqueous solution.

5. Studies with sludge incinerators on the effect of various additives other than iron, aluminum, or calcium salts on trace element leaching with a view toward a less easily leached sludge should be conducted.

SECTION 4

REVIEW OF THE LITERATURE

MINERALOGICAL AND PHYSICAL PROPERTIES

Ashes from municipal sludge incinerators are composed chiefly of aluminum, calcium, iron, phosphorus, silicon, sulfur and smaller amounts of trace elements[9,10]. Loss on ignition is reported to vary between 0.3 percent to 15.13 percent with most losses less than 3 percent[9,11]. The forms of the major matrix elements is a matter of some uncertainty. It is common to report concentrations as the respective oxides of each element, however available x-ray diffraction studies suggest that this is not always the case. Gray and Pennessis[9] report finding evidence for SiO_2, but also for several mixed crystalline compounds including calcium aluminum silicates, calcium phosphate, calcium aluminate, iron magnesium oxide, and calcium carbonate. The identification of $CaCO_3$ may be spurious; Williams et al.[12] performing x-ray studies on ash produced at different temperatures, report the disappearance of calcite between 450°C and 650°C and its replacement by calcium oxide (lime). The normal maximum temperatures reached in modern incinerators are 800-900°C[4]. Other minerals identified by Williams et al. included feldspar, anatase (TiO_2) at 450°C, anhydrite ($CaSO_4$) and hydroxylapatite. Interesting changes which occurred at temperatures above 800°C were the disappearance of anhydrite (as sulfur is volatilized), anatase (as it condensed with lime forming perovskite, $CaTiO_3$), and lime as it contributed to the formation of hydroxylapatite. Hydroxylapatite assumed a greater dominance as temperatures increased over the range 650°C to 900°C indicating a higher degree of crystallinity. Gulbrandsen et al[13] confirm the presence of hydroxylapatite and feldspars, and also report finding rutile, corundum, talc, muscovite, zircon, garnet, cristobalite, and hematite, the latter two most likely having been formed during the incineration process.

The dominant minerals on the surface of the ash affect the surface area. Measurements of specific surface area for three sludge ashes ranged from 3.4 m^2/g to 11.5 m^2/g[14]. Many of the surface minerals exhibit a high water solubility such that the surface area of the material in contact with water would vary considerably from these values.

Specific gravities of several ashes ranged from 2.44 to 2.96[9,15], while bulk densities varied from 0.48 to 0.80 kg/ℓ^3 (30-50 $lbs./ft^3$)[9,11].

No studies related to the surface charge characteristics (colloid chemical properties) of sludge ashes could be found.

TRACE ELEMENT CONCENTRATIONS

The definition of what constitutes a trace element is not always clear. Logically it should include those elements present in a given sample in amounts less than a specific concentration -- perhaps 1% by weight. In practice various transition metals, usually referred to as heavy metals, are included in most trace analyses even if, on occasion, they are found at levels higher than "trace" quantities. Many of these have industrial significance, are often toxic, and their presence in the environment has historically been of interest. They would include Ag, Cd, Co, Cr, Cu, Hg, Ni, Pb, and Zn. Because the aqueous solubilities of many of these elements is often controlled by oxides of iron and manganese, these elements are also included if present. To this list are often added the metalloids As and Se, and the alkaline earths Ba and Be. Using these elements as a reference base, a search of the literature found various tabulations of bulk trace element concentrations for different sludge ashes. These are summarized in Table 1. It is worth noting that, in comparison with similar data for raw, digested, and dewatered sewage sludges, the comparable data base for sludge ashes is not as extensive.

Strict comparisons among the values listed in Table 1 are not advisable because of the individual variations in sampling procedures, digestion techniques, and analytical methods. For example, Farrell and Salotto estimate the accuracy of spark-gap emission spectroscopy at no better than ± 30 percent. With respect to digestion procedures, it is surprising that only Gabler and Neylan report using HF for dissolution of silicates, which comprise between 15 and 60% of the ash material[9].

In spite of these variations, it is possible to gain an acceptable evaluation of elemental concentration ranges in sludge ash. Several aspects of the data are worth expanding upon. Inspection of data on metals in raw and digested sludge[23] (on a wt/wt basis) indicates the concentration effect for all metals measured in sludge ash, except mercury, is close to a four-fold increase, as would be expected for municipal sludges with approximately 25 percent fixed solids. Mercury and selenium compounds are, of course, quite volatile even at relatively low temperatures[24]. In comparison with natural crustal abundances, the metals are two to three orders of magnitude higher for sludge ash[25,26,27]. Thus sludge ashes may represent an important source of metals to the environment. Furr *et al.*[22] report some bioconcentration of cadmium, cobalt, copper, and zinc in cabbage grown on soil ammended with selected sludge ashes. The availability of trace elements will depend on several factors, particularly the major elemental reservoir concentrations of Fe, Al, and Ca. Furr and co-workers point out the dependency of these concentrations upon the prior addition of lime, alum, and ferric chloride to the wastewater treatment process.

TABLE 1. TRACE ELEMENT CONCENTRATIONS IN SLUDGE ASHES (all values mg/g dry wt)

ELEMENT	DIOSADY[1] (16, 17)	FARRELL & SALOTTO[2] (10)	GABLER & NEYLAN[3] (18)	GULBRANDSEN[4] et al (13)	OLIVER & CAREY[5] (19, 20)	SWEENEY[6] (21)	FURR et al[7] (22)
Ag	n.a.	n.d. - 0.36	.024 - .280	0.68	n.a.	n.a.	n.a.
As	n.a.	n.a.	n.d.	n.a.	n.a.	n.a.	.0009 - .022
Be	n.a.	n.d.	n.d.	.005	n.a.	n.a.	n.a.
Ba	n.a.	n.d. - 3.0	1.2 - 8.7	2.60	n.a.	n.a.	.295 - 5.44
Cd	n.a.	n.d. - 0.58	.006 - .096	.068	.01 - .05	n.a.	.0007 - .127
Co	n.a.	n.d.	.011 - .290	.360	n.a.	0.03	.0082 - .069
Cr	3.12 - 4.60	0.4 - 5.9	.052 - 3.60	1.45	n.a.	0.80	.411 - 9.70
Cu	1.77 - 2.25	0.9 - 6.0	.21 - 2.20	7.40	0.6 - 30	1.11	.513 - 3.23
Fe	29.9 - 39.3	8.7 -27.4	15.9 - 151.6	48	43 -146	40.67	17.66 - 91.72
Hg	n.a.	.003- .0055	n.d.	n.a.	n.a.	n.a.	.00004- .00018
Mn	n.a.	0.18 - 1.2	.26 - 2.40	.68	n.a.	0.84	.342 - 3.095
Ni	0.252 - 0.572	n.a.	.056 - 1.50	1.7	0.3 - 0.8	0.22	.04 - 4.20
Pb	1.51 - 2.60	0.8 - 6.9	.16 - 1.80	1.8	0.2 - 3.6	0.24	.082 - 1.33
Se	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	.00004- .0009
Zn	3.09 - 6.99	0.4 - 8.4	.57 - 5.00	.27	1 - 18	1.85	.40 - 8.00

n.a. = not analyzed; n.d. = not detected

[1]Range for 10 samples for one plant; aqua regia digestion - AAS.

[2]Range for composite samples collected from 7 plants; spark-gap emission spectroscopy.

[3]Range for grab sample from 14 plants; HF-$HClO_4$ digestion - AAS.

[4]Average of two samples at different times from one plant; digestion procedure not given, various analytical methods.

[5]Range of samples from seven plants; digestion and analysis procedures not given.

[6]Average of 5 samples from one plant; H_2SO_4-$HClO_4$-HNO_3 digestion - AAS.

[7]Range of values for single samples from 10 plants; neutron activated and other analytical methods.

The lack of data for arsenic and selenium is surprising since both of these elements have been identified as being comparatively transportable in the environment[24,28]. Gabler and Neylan reported As to be less than their detection limit of 20 µg/g for 14 ashes analyzed. The work of Furr et al. gives a range of As of 0.9 to 22 µg/g. It can be argued that such low concentrations diminish the relative importance of this element. The uses of arsenic, however, in industries such as pesticide manufacture and metallurgical alloys are not as widespread as those which make use of other metals. It is possible that arsenic is present at significant concentrations for selected sludge ashes.

PARTICLE SIZE EFFECTS

The ash residue from sludge incinerators consists of a gradation of particle sizes. From the point of view of leachate composition for ash materials, the size distribution (i.e., surface area distribution) can have important effects on surface reactions. It is legitimate, therefore, to inquire into what is known about size fractionation of sludge ash.

Comparison of particle distribution for sewage sludges as they are processed through anaerobic digestion reveals a breakdown of large particles greater than 100 µm)[29,30]. The size distribution is approximately log-normal. Upon incineration, however, Dewling[11] indicates a bimodal distribution is created with 30-80 percent of the particles in eight sludge ashes having particle diameters greater than 150 µm, and lower maxima containing between 25 and 60 percent of the particles, with geometric mean diameters between 11.5 and 28 µm. The mass distribution of trace species was also bimodal, with the majority of metals contained in the smaller diameter size fraction due to preferential partitioning to smaller particles during combustion. Reasons for this are unclear. Dewling disputes the volatilization-condensation model which has been used to describe a similar phenomenon for coal fly ash[31], insisting that temperatures in incinerators are not high enough (800-900°C) to volatilize most metal forms. This conclusion is open to question, and other explanations have merit.

One of the interesting aspects of Dewling's work was the discovery of a distinct difference in particle size distribution between multiple hearth and fluidized bed incinerators. Although both are bimodal, the abrasive action afforded by the sand used in fluidized bed incinerators yields a larger amount of material in the smaller size fraction than multiple hearth incinerators.

CHEMICAL LEACHING TESTS

Gray and Pennesis[9] report the overall solubility of sludge ash in distilled water to be between one and two per cent. Most researchers find an alkaline reaction in water, as might be expected from the dissolution of

surface lime deposits; however Dewling[11] reports several ashes which resulted in acid reactions as low as pH 5.1. Theis and Wirth[24] observed similar behavior for coal fly ash and related the pH to the relative amount of lime and amorphous iron oxides, the latter producing protons through hydrolysis reactions.

Several researchers have investigated the leaching characteristics of sludge ashes from the point of view of establishing a procedure for detoxifying the ash prior to disposal and/or recovering trace elements from the ash. Strong acid leaches, with 25 to 70% sulfuric acid, were particularly effective in recovering trace species although a variety of other leaching agents were also used. A qualitative summary from the literature is given in Table 2. Unless otherwise indicated, the leaches of Table 2 were of the single batch variety with solids concentrations of 5 to 80 grams per liter.

Certain of the leaching agents of Table 2 were used for rather selective purposes. For example NaCN was used by Gabler and Neylan[19] in an attempt to maximize the solubiliztion of gold which was contained in sludge ash from Palo Alto, California. Oliver and Carey[20] used basic ammonia leaches to try to separate certain metals on the basis of their tendencies to form soluble amine complexes.

The work of Diosady[16,17] displays the greatest variation in types of leaching agents used. Oxidative (persulfate, H_2O_2), complexometric (EDTA, detergent -- pyrophosphate), basic (NaOH), weak acid (acetic, H_3PO_4, citric acid), and strong acid leaches were made. The strong acid extractions of Diosady plus that of Gabler and Neylan and Oliver and Carey were investigated, as indicated previously, in order to maximize trace element release. This is important information and suggests methods of sludge detoxification which may become feasible at some time, however these extractions tell little about trace element leaching potential land transport availability in a disposal site. In this respect the less chemically vigorous extractions are, perhaps, more useful. Unfortunately the ionic concentrations of extractant in Diosady's preparations are so high that it is not possible to distinguish between metals which are loosely held on surface sites (adsorbed), those which are more strongly bound as discrete or mixed precipitates, and the surface elemental associations which exist. All of these factors are useful in defining the release characteristics of trace elements under specific conditions. In addition, Diosady made no analyses for cadmium, since it is not present in amounts considered economically recoverable. The variety of leaching agents used by Diosady are probably best summarized in terms of their "solvating power" as given in Table 3.

It is difficult to make definitive statements from Table 3. The sodium carbonate fusion followed by acid leach gives the greatest release for all metals, except Ni. This procedure converts large amounts of the metals to a readily soluble form. Nickel forms the strongest complex, among the metals studied, with EDTA. The fact that so much Ni was readily solubilized by EDTA and the detergent suggets that it is in a surface leachable form, but is bound tightly enough so that strong acids are able to solvate

only a portion. Leaching agents which contain sulfate (sulfuric acid and persulfate) are poor extractants for lead, due primarily to the insolubility of $PbSO_{4(s)}$. Although few statements regarding the form of trace elements on sludge ash can be made, the work of Diosady indicates that differential extractive preparations can be made with sludge and that this is a viable investigative technique. The work of Gabler and Neylan[18] and Oliver and Carey[19,20] with strong acid extractions generally support the findings of Diosady that dilute sulfuric acid is the most economical method of recovering metals from sludge ash.

An interesting finding by both Gabler and Neylan and Williams et al.[12] relates to the relative extractability of metals from ash formed at different combustion temperatures. Both indicate a gradual decrease in metals removed, Gabler and Neylan with 25% H_2SO_4, Williams et al. with .033 EDTA, as combustion temperature increases. This may reflect the greater degree of crystallinity and lower surface area of the ash minerals at higher temperatures. It should be noted, however, that the amounts of metals released even at the highest temperature (800-900°C) were greater than comparable studies show for uncombusted sludge[32]. Raw sludge may contain metals in the form of insoluble sulfides which are unstable at higher temperatures.

The batch leaching studies of Sweeney[21] are noteworthy because of the different procedure followed. Serial batch leaches in distilled water adjusted with HNO_3 to pH 5.3 and 4.0 were carried out for as many as 40 consecutive washings. This procedure, evidently by coincidence, is similar to the International Atomic Energy Agency (IAEA) leachate test which consists of a daily serial batch leach for periods of up to 90 days[33]. This test has been widely used for radioactive wastes and appears to be gaining acceptance for testing on other materials. Sweeney's results show that the major portion of most trace elements leach into solution within 15 batches. The rates of leaching were Cr > Cu > Ni > Mn > Fe. Chromium in particular leached rapidly at pH = 4.0 reaching an initial concentration in batch #1 of 140 µg/ℓ. Initial Cu and Ni were 95 µg/ℓ and 100 µg/ℓ , respectively. In column studies, the initial concentrations of Cr, Cu, and Ni were much higher, reaching 6400 µg/ℓ, 157 µg/ℓ , and 216 µg/ℓ, respectively. This is probably due to the higher initial solid solution ratio. Unfortunately no measurements were made for cadmium.

WET CHEMICAL EXTRACTION PROCEDURES

The use of chemical extractants to study the surface chemical properties of solid materials is a common investigative tool. Soil scientists were among the first to systematize various agents and procedures and document their effects. More recently, standardized leaching tests have been proposed as a means of assessing the availability of surface constituents to the environment. Several non-standard procedures were reviewed above. The major focus of these was heavy metal recovery from sludge ash. In only a few cases was this economically feasible. In this section, many more extraction agents and procedures will be reviewed.

TABLE 2. LEACHING AGENTS USED FOR SLUDGE ASH

AGENT	DIOSADY (16,17)	GABLER & NEYLAN (18)	OLIVER & CAREY (19,20)	SWEENEY (21)	WILLIAMS ET AL (12)
Strong Acids	X[1]	X[6]	X[8]	X[9]	X[11]
$Na_2S_2O_7$	X[2]				
Distilled Water	X[3]			X[10]	
EDTA	X[4]				X[12]
9% H_2O_2/70% H_2SO_4	X				
50% Citric Acid	X				
Conc. Acetic Acid	X		X		
Conc. H_3PO_4	X				
30% Household Detergent	X				
40% NaOH	X				
Persulfate Fusion	X[5]				
Na_2CO_3 Fusion	X[5]				
NaOH Fusion	X[5]				
NH_4OH, pH = 13			X		
$NH_4OH(NH_4)SO_4$			X		
NaCN (0.024M)		X			
NaCN (.59M)		X			
HCl/.059M NaCN		X[7]			

[1]Variable concentrations of sulfuric (50% to 90%) and nitric (50% to 10%) acid mixture, H_2SO_4 alone (conc. to 10%), and concentrated HCl.

[2]Variable concentrations of $Na_2S_2O_7$ alone and in mixtures with 50% H_2SO_4.

[3]pH uncontrolled

[4]1% solution of unspecified salt of EDTA.

[5]All fusion residues were leached with acetic and hydrochloric acid in two stages.

[6]Batch leachates with H_2SO_4 (primarily at 25%), 25% HNO_3, and concentrated HCl. For H_2SO_4, both concentration of acid and temperature of extraction (45°C - 110°C) were varied. A three stage batch counter-current leaching procedure is proposed.

[7]Initial leach with HCl followed by NaCN.

[8]Variable concentrations of H_2SO_4, HNO_3 and HCl. A counter-current leach is also proposed.

[9]pH 4.0 titration of sludge ash (250 g/) with 1.0 N HNO_3. Titration carried out for 96 days to determine buffer capacity of ash.

[10]Consecutive batch leaches in distilled water at pH 5.3 and 4.0. Carried out for 45 batches. Also column leach continued for 6 to 7 bed volumes.

[11]Boiling 20% HCl.

[12]0.033 M EDTA at pH = 5.

TABLE 3. SOLVATING POWER OF LEACHING AGENTS FOR METALS IN SLUDGE ASH ARRANGED IN DECREASING ORDER

Cr	Cu	Fe	Ni	Pb	Zn
Fusion/HCl>	Fusion/HCl>	Fusion/HCl>	1% EDTA>>	Fusion/HCl	Fusion/HCl>
$Na_2S_2O_7/H_2SO_4$>	H_2SO_4/HNO_3>	$Na_2S_2O_7/H_2SO_4$>	Fusion/HCl>	Aq. Reg.>	$Na_2S_2O_7/H_2SO_4$>
H_2O_2/H_2SO_4>	100% H_2SO_4>	dil H_2SO_4>	dil H_2SO_4>	conc. H_3PO_4>	H_2O_2/H_2SO_4>
	dil H_2SO_4				
H_2SO_4/HNO_3>	$Na_2S_2O_7/H_2SO_4$>	conc. HCl^-	30% $Na_2S_2O_7$-	conc. HCl>	H_2SO_4/HNO_3>
dil H_2SO_4>	Aqua Regia >	Aq. $Reg.^-$	$Aq.Reg.^-$	conc. HNO_3>	dil H_2SO_4>
conc. HCl>	30% $Na_2S_2O_7$>	conc. H_3PO_4>	conc. $H_3PO_4^-$	1% EDTA>	conc. H_2SO_4>
Aqua Regia>	10% $Na_2S_2O_7$>	H_2SO_4/HNO_3>	conc. $H_2SO_4^-$	50% CIT>	$Aq. Regia^-$
conc. $H_3PO_4^-$	conc. HCl>	30% $Na_2S_2O_7$>	DET >	dil $H_2SO_4^-$	30% $Na_2S_2O_7$>>
30% $Na_2S_2O_7$>	conc. HNO_3>	20% $Na_2S_2O_7$>	H_2SO_4/HNO_3^-	conc. HAc -	conc. HCl>>
conc. H_2SO_4>	conc. H_3PO_4>	50% CIT >>	$Na_2S_2O_7/H_2SO_4$>	$Na_2S_2O_7/H_2SO_4$>	40% NaOH-
20% $Na_2S_2O_7$>	1% EDTA >	conc. HNO_3^-	conc. HCl>	conc. H_2SO_4 >	20% $Na_2S_2O_7$>
conc. HNO_3 >	50% CIT >	H_2O_2/H_2SO_4 >	20% $Na_2S_2O_7$-	H_2O_2/H_2SO_4 >	50% CIT -
DET >	H_2O_2/H_2SO_4 >	1% EDTA >>	conc. HNO_3 >	DET >	conc. H_3PO_4>
50% CIT >	conc. HAc >	conc. HAc >	H_2O_2/H_2SO_4 >	H_2SO_4/HNO_3>	conc. HNO_3>
1% EDTA >	DET >	DET -	50% CIT>	5% $Na_2S_2O_7$>	1% EDTA >
conc. HAc >	40% NaOH >	conc. H_2SO_4 >	conc. HAc >	30% $Na_2S_2O_7$ -	DET -
40% NaOH >	D.W.	40% NaOH >	40% NaOH >	40% NaOH >	conc. HAc >
D.W.		D.W.	D.W.	D.W.	D.W.

The most logical way of classifying extracting agents is by their primary chemical interaction with the solid surface. This has been done for several of the more common extractants, along with their major application in Table 4. Few of these extracting agents have been applied specifically to wastewater sludge ash; those which have were reviewed above. It should be noted that important parameters, such as contact time and temperature of the medium, are not included in Table 4.

Several of the extracting agents in Table 4 have been incorporated into recommended tests for leachate asessment by certain agencies. Four of the more widely used of these are gathered in Table 5. The major criticism of procedures such as those in Table 5 is that they are intended for application to virtually any solid waste material. Ham et al.[34] have shown the difficulty of devising only one test for use with widely different waste materials. Even for testing with one waste, Bause[35] found only the ASTM-B Method to yield acceptable reproducibility in a comparison among the first three tests of Table 5.

TABLE 5. COMMON SINGLE-REAGENT LEACHATE TESTS

Agency	Test Name	Major Extracting Agent	Ref.
U.S. EPA (Office of Solid Waste)	Extraction Procedure (related to RCRA testing)	Acetic Acid	38
ASTM	Method A - Water Shake Extraction Procedure	H_2O	37
ASTM	Method B - Acid Shake Extraction Procedure	Acetic Acid	37
IAEA[a]	Leach Test	H_2O	33

[a] International Atomic Energy Agency

The selectivity of many of the reagents in Table 4 can be greately enhanced through their incorporation into serial extraction procedures wherein the solid material is sequentially contacted with one extractant at a time. Such schemes are often referred to collectively as "Gibbs fractionations" after the work performed by R.J. Gibbs with river particulate transport phases[36]. It is possible to gain significant information about heavy metal associations with specific extractable phases or similar mineral assemblages. This knowledge can be useful in assessing the release potential of toxic species in a disposal environemnt. A major advantage of this approach is that the type and order of extracting reagents can be altered for application to a specific material. Thus,

TABLE 4. COMMONLY USED EXTRACTING AGENTS

EXTRACTING AGENT	PRIMARY CHEMICAL INTERACTION	MAJOR APPLICATION	REFERENCES
H_2O	Hydration	Leachate Assessment	40, 41, 42
Acetic Acid	Acidic	Leachate Assessment	3, 20, 42
H_2CO_3	Acidic	Leachate Assessment	43
H_3PO_4	Acidic	General Metal Recovery	20
Citric Acid	Acidic	General Metal Recovery	20
Dilute Strong Mineral Acids	Acidic	General Metal Recovery HCl-Dissolution of Metal Oxides	20, 44
Conc. Strong Mineral Acids	Acidic	Dissolution of Major Mineral Forms (except silica) General Metal Recovery	20, 29, 31
HNO_3-HCl-HF	Acidic	Measurement of Total Concentration	45
NaOH	Basic	Dissolution of Polar Organic Fraction Dissolution of Fe, Al Oxides	46, 47, 48
$BaCl_2$, $MgCl_2$, $CaCl_2$ KNO_3, KF, NH_4Ac	Solvating Power due to high ionic strength	Exchangeable and Physically Adsorbed Species	27, 46, 49, 50
EDTA	Complexometric	Exchangeable and More Strongly Bound Metals Dissolution of carbonate Recovery of Metals from Organics	17, 20, 27, 51
$Na_4P_2O_7$	Complexometric	Recovery of Metals from Organics	27, 52, 54
Ammonium Oxalate (acid)	Complexometric	Dissolution of Amorphous Oxides of Al and Fe	49, 53
Citrate-Dithionite-Bicarbonate	Reductive	Dissolution of Fe and Al Oxides	49, 53, 54, 55
Hydroxylamine Hydrochloride	Reductive	Dissolution of Mn Oxides	56
H_2O_2	Oxidative	General Metal Recovery Organic Digestion	20, 52
Persulfate	Oxidative	Dissolution of Major Mineral Forms	20
Methylene Chloride	Dissolution of Most Organics	Recovery of Trace Organics	57
Dimethylsulfoxide (DMSO)	Dissolution of Most Organics	Recovery of Trace Organics	58
Ethanol	Dissolution of Polar Organics	Bioavailability Studies	44

although this general method remains popular in work done with suspended river particulates[41,46], it has been successfully applied to lake and marine sediments[51,52], stormwater solids[53], anaerobically digested sludge[7], raw wastewater sludges[45], particulate aerosols[54], and, of course, soils[43,45].

ORGANIC CONTENT OF SLUDGE ASH

As indicated, organic residues in sludge ash have been reported to comprise 1-15 per cent of the total ash by weight. Wells et al.[56] studied stack emissions from a sludge incinerator and reported the presence of such organics as ethyl and methy ethyl benzene, toluene, styrene and other aromatic compounds, as well as saturated and unsaturated hydrocarbons. Organics occurring in incinerated waste can also include chlorinated hydrocarbons (such as those from pesticides in agricultural land runoff and polychlorinated biphenyls (PCB)) in addition to the more highly condensed polynuclear aromatic hydrocarbons (PAH).

While Furr and his co-workers[22,57] discovered PCBs in municipal sewage sludges, neither they nor Farrell and Salotto[10] detected them in sludge ash. However, they proposed that the disappearance of PCBs was not completely due to thermal decomposition but also to the adsorption of volatilized condensates onto particulates that escaped collection and were emitted from the incinerator stack. This potential for PCB survival was supported by Eiceman et al.[58] and Suzuki et al.[59], both of whom established the presence of PCBs in municipal refuse ash. Other research by Komamiya and Mirosaka[60] indicated that at temperatures much higher than those reached in a conventional furnace (2900°C) PCBs were effectively destroyed. These investigators provided an explanation of the high temperatures necessary for the destruction of some PCBs with the statement that those molecules containing four or more substituted chlorines can be considered inflammable.

Large amounts of PAH were also detected in municipal refuse ash[58]. Davies et al.[61] reported the presence of polycyclic aromatic hydrocarbons adsorbed onto ash particles from municipal incinerators which were maintained at temperatures between 800°C and 1090°C. Harrison et al.[8] stated that PAH are formed during the combustion process. In general, the lower the temperature the greater the concentration of PAH formed. Hughes et al.[62] explained that polycyclic organic materials are primarily the result of incomplete combustion of organic substances within the incinerator. Hot and cold spots can occur inside an incinerator, especially during times of start-up and change, and would account for such incomplete combustion and retention or production of potentially harmful organics. The largest quantities of PAH leaving the incinerator were found in the ash residue (as opposed to quench waters or stack gases)[61].

THE AMES TEST

Certain procedural details of the Ames test will be discussed in Section 5 of this report, however background information and explanatory remarks will be presented here.

Designed by Dr. Bruce N. Ames of the University of California, the test is a bacterial/mammalian microsome assay which utilizes histidine auxotrophs of the microorganism Salmonella typhimurium in conjunction with active enzymes. In the presence of mutagens and potential carcinogens, the specially-constructed Salmonella strains revert to prototrophy. This method of screening environmental chemicals for possible carcinogens takes only two days to complete, is inexpensive and sensitive, and has enjoyed widespread popularity since its inception in 1973[63]. A review of industrial, governmental and academic laboratories indicated that over 2,000 performed the Ames assay, testing an estimated 2,600 substances[64]. In an assay of 300 chemicals labeled by previous test results as "carcinogens" or "noncarcinogens", McCann et al.[65] reported a 90 percent correlation between mutagenicity in this test and carcinogenicity in animal studies.

The Ames test is based on measuring the damage done to the DNA, the genetic material, of the bacterial strains. The hypothesis that a carcinogen's mode of action is in its damage to an organism's DNA has received strong support in the scientific community. McCann and Ames[66] provided evidence in agreement with this belief, stating that: it was consistent with facts of cancer biology and studies on the genetics of cancer; mutations were known to alter cell regulation, and one of the characteristics of a cancer cell is a heritable change in its regulation; many human mutants with defects in the DNA repair system have proven very cancer-prone[67]; and there exists a wide variety of carcinogens -- from metals and asbestos to radiation -- whose only common feature appears to be the ability to damage DNA.

The recommended test battery for the Ames assay for general mutagenicity testing consists of five strains: TA 98, TA 100, TA 1535, TA 1537, and TA 1538[68]. All were derived from the same wild-type LT-2 parent Salmonella strain[63], and each has its particular histidine marker (pertaining to its mutant condition as an auxotroph). Herbold and Buselmaier[69] reported that TA 1535 and TA 100 underwent a missense mutation in the histidine operon, while TA 1537 was thought to have resulted from the insertion of a guanine/cytosine (G/C) pair. Two base pairs, (G/C, C/G) were inserted to create the final main strain TA 1538 and TA 98. TA 1538 and TA 100 were designed to detect base pair substitutions (TA 100 being isogenic to the former except for its possession of an R factor), and strains TA 1537, TA 1538, and TA 98 were designed for frameshift detection (TA 98 isogenic to TA 1538 but for the additional R factor).

To increase the sensitivity of the screening test Ames added several alterations to the special Salmonella strains. The first was an excision repair mutation which eliminated the bacteria's capacity to correct any variations it may have detected in its genetic code. The lipopolysaccaride surface coat of the organism was weakened through elimination of portions of the polysaccharide chain. This increased permeability of the protective coat to chemical penetration[70], thereby causing the bacterial cell to more closely resemble a mammalian cell.

Finally, Ames introduced an R (resistance) factor via the pKM101 plasmid into the genetic information of the tester strains. A small piece of foreign DNA, the plasmid contains a resistance marker for the antibiotic ampicillin and is believed to make the bacteria's replication of DNA more error-prone[71], as well as much more sensitive to some chemicals[72].

Ames included the use of enzymes in the test to make the assay complete. Many environmental chemicals are considered harmless until they are changed to their active form by a metabolic conversion process[73]. The biotransformation of such chemicals in mammals has been attributed to microsomal liver enzymes. Enzymes from rat liver extract convert procarcinogens to their active metabolites and this was the basis for the use of mammalian microsomes in the Ames assay[74]. Inclusion of these enzymes affords the test a certain amount of relevance to humans, since it permits simulation of the effect human metabolism has on ingested or inhaled chemicals in activating them to mutagenic forms within the human body.

The theoretical basis of the Ames test revolves around bacterial reversion and growth. The test chemical is combined with a mutant bacterial mix, the liver enzyme mix if desired, and a very small amount of histidine. This trace amount is insufficient for the formation of colonies from the auxotrophic tester strains, but it allows enough growth for the expression of mutations.

A back mutation restores the normal sequence in the bacterial genetic code, so revertant bacteria will again be capable of producing an internal supply of histidine. It is only these revertants that can form colonies on the test plates, which are then scored and compared to the known, low spontaneous number of reversion expected. In the presence of a mutagen, sometimes reportedly in only nanogram amounts[75], the elevated number of revertant colonies reflects the test compound's ability to induce mutations in the tester bacteria.

SECTION 5

EXPERIMENTAL METHODS

GATHERING OF SAMPLES

Requests for sludge ash and dewatered sludge samples were made to 12 wastewater treatment facilities in late 1979. Ten facilities returned ash and sludge samples that were collected at approximately the same time. Treatment plants that responded were: Detroit, Michigan; Green Bay, Wisconsin; Independence, Missouri; Indianapolis, Indiana; Jersey City, New Jersey; Montrose, Michigan; Parsippany, New Jersey; Pontiac, Michigan; Port Huron, Michigan; and Waldwick, New Jersey. Data were also provided on their wastewter treatment and sludge treatment and incineration processes. This information is provided in the next section of this report.

The sludge ash and dewatered sludge samples were stored at room temperature in sealed containers. A portion of each sample was placed in an oven at 105°C in preparation for further analysis.

CHARACTERIZATION OF SAMPLES

The general appearance of the ash was documented with the aid of photomicroscopy. A small amount of ash was centered on a glass slide and a drop of immersion oil was added before sealing with a glass coverslip. An Olympus camera attached to a Zeiss light microscope and loaded with Kodacolor C-135 film was used for this stage of the ash characterization. Photomicrographs were taken with both reflected and transmitted light, in magnifications ranging from 100X to 400X.

A scanning electron microscope (SEM) was used to observe the microstructure of the incinerated waste samples. A Cambridge Steroscan 600 SEM equipped with an Ilex camera loaded with type 665 positive/negative Polaroid land film was used. Sample preparation involved covering a platinum stud with double-backed tape and sealing the edges with silver paint, then dipping the stud upside-down onto an ash sample and tapping off the excess. Ash-covered studs were then coated with either carbon or gold to reduce charging of the sample (caused by the impact of high-speed electrons on the sample). Carbon coating was done with a Commonwealth Scientific Mini-Coater; gold coating was achieved in a CD 515 Denton Vacuum system. Generally, a spot size of 2 or 4 and an electron voltage of 1.5 kv were used in observing the samples with magnification of 20X to 3000X.

A second scanning electron microscope with an X ray fluorescence (XRF) attachment was used for qualitative determination of the elemental composition of ash samples. The Super II SEM (replaced by an International Scientific Instruments - 60 A SEM) with attached PGT (Princeton Gamma Tech) Delta computer system permitted association of X ray fluorescence peaks (emitted from the ash and displayed on a screen) with the element responsible for that peak (determined by characteristic energy levels). The Super II and ISI-60A SEMs were both generally operated at 20 kv with counting rates around 1,000 and magnifications from 100X to 200 X. The studs prepared for the Cambridge 600 SEM were also used in the Super II and ISI-60A electron microscopes.

MEASUREMENT OF pH

Hydrogen ion concentration was determined according to Jackson[43]. Twenty five milliters of double distilled water was added to 10 g of dry sludge or sludge ash. The mixture was constantly stirred by means of a magnetic stirring bar for 20 minutes. Equilibrium pH was measured with a glass electrode pH meter.

DRY WEIGHT

The dry weight or solids content of the dewatered sludge samples was determined according to Standard Methods[76]. A 30 gram sample of dewatered sludge was transferred to a preweighed crucible, placed in an oven at 105°C overnight, cooled in a desiccator and weighed again.

Since sludge ashes are naturally moisture free immediately after incineration, dry weight was not determined.

VOLATILE MATTER

Volatile matter of the sludge ash (and dewatered sludge samples) was determined according to Standard Methods[76]. A sample was dried overnight at 105°C and cooled in a desiccator. Thirty grams of material was transferred to a preweighed crucible, weighed, and placed in a muffle furnace at 550°C for one hour. The crucible and contents were then cooled in a desiccator, and weighed again.

ELEMENTAL ANALYSIS

Complete digestion of the sludge ash was achieved in a teflon Parr bomb digestion vessel by the hydrofluoric acid-aqua regia procedure of Bernas[40]. This procedure was also used for digestion of dewatered sludge samples. After digestion each sample was filtered with a 0.45 μm Millipore filter. Elemental concentrations were determined by flame or flameless atomic absorption spectroscopy (AAS) with background correction. When determining total calcium, lanthanum chloride was added (final concentration 1%) to eliminate interferences from calcium sulfate and calcium phosphate

complexes[77]. Analyses for arsenic and selenium were measured by flameless AAS using the nickel complexation method as described by Ediger[78].

When analyzing heavy metal concentrations in the dewatered sludge samples 30% hydrogen peroxide was added (final concentration 2%) to oxidize the organics and minimize background absorption[17,46].

SINGLE-REAGENT EXTRACTION TESTS

There are two basic approaches to extraction testing of a soli material. One involves using a single major reagent, the other utilizes reagents applied in a sequential procedure. Both of these approaches were used in this study. In this section, the single reagent tests used are briefly reviewed.

ASTM Method A - (ASTM-A) Water Shake Extraction Procedure -- This extraction method utilizes distilled water as the extracting agent[33]. A volume of 40 mℓ of double distilled water was added to ten grams of dry ash to produce a water to solid mass ratio of 4 to 1. Agitation was provided to produce constant movement of the mixture by a mechanical platform shaker. The extraction flask was sealed and closed to the atmosphere for the duration of the 48 hour test.

The aqueous phase was separated from the solid phase by filtration through a 0.45 μm membrane filter. The extract was acidified with a 0.2 mℓ of concentrated nitric acid and diluted to 100 mℓ with double distilled water. This test was performed on all ten ashes but not on the dewatered sludges.

U.S. EPA Extraction Procedure -(EP) -- This extraction procedure employs the use of 0.5 M acetic acid as the extracting agent[38]. A volume of double distilled water was added to 5 grams of dry ash to produce a solution to solid ratio of 16 to 1. A jar test apparatus was used to insure that the solid sample was continuously brought into contact with well mixed extraction fluid. The pH of the mixture was adjusted with the acetic acid to 5.0 $\pm$ 0.2 at regular intevals throughout the 24 hour extraction period. Addition of acetic acid was limited to 4 mℓ of acid per gram of ash for the 24 hour period. If the pH was not between 4.8 and 5.2 after the 24 hour period then pH adjustment continued at one hour intervals for a period not less than 4 hours. The mixture was centrifuged, filtered through a 0.45 μm membrane filter, acidified with0.2 mℓ of concentrated nitric acid, and diluted to 100 mℓ with double distilled water.

The EPA extraction procedure was performed on both the sludge ash and dewatered sludge samples.

International Atomic Energy Agency (IAEA) Leach Test -- The International Atomic Energy Agency has established a standard method to measure the leaching rate of radioactive components from a solid waste[33]. The extracting agent is distilled water. In contrast to the ASTM Method A

Extraction Procedure, this test is conducted without agitation of the solid aqueous system, and involves successive batch equilibration periods. It is the only extraction test used in this study which incorporated a long-term time factor.

The IAEA test was performed on 25 grams of dry ash with a solution to solid ratio of 12 to 1. The pH of the extraction fluid was not adjusted. The leachate was decanted and filtered through a 0.45 μm membrane filter each day for the first week and every seventh day thereafter for 21 weeks. The pH was measured and recorded. The same volume of double distilled water was added to the sludge ash very slowly along the side of the beaker to prevent disruption of the ash surface.

Samples were acidified with concentrated nitric acid and stored at 4°C. This test was performed on the sludge ashes only.

SERIAL EXTRACTION PROCEDURE

Most studies dealing with metals in wastewater sludge or ash discuss total concentrations with no attempt to evaluate the various forms in which metals might exist. It is possible to gain important information about heavy metal association through the use of specific extracting reagents. This knowledge can be useful in assessing release potential in a disposal environment.

As explained in Section 4, a number of reagents including acids, bases, salts, and complexing agents are useful in extracting metals. Laboratory studies were conducted to develop a sequential fractionation scheme which provided more detailed information on the forms of metals on incinerator ash.

The final procedure, summarized in Table 6 and given in greater detail in Appendix A, consisted of successive extractions in calcium chloride (easily exchangeable forms)[55], sodium pyrophosphate (alkaline extraction selective primarily for aluminum)[79], acid ammonium oxalate (amorphous iron and aluminum oxides)[47], citrate-dithionite-bicarbonate (other iron and aluminum oxides)[80] and hydrofluoric/aqua regia (complete dissolution)[40]. Since some of the sludge ash samples were from wastewater treatment facilities which add iron and/or aluminum salts, selective reagents were used to investigate the possibility that heavy metals might be partitioned into the iron and aluminum fractions of the ash. It is well known that ferric and aluminum hydroxides are excellent metal scavengers[81].

Heavy metal concentrations of the serial extractions were determined by flameless atomic absorption spectrophotometry. Scanning electron microscopy with x-ray fluorescence (XRF) was used to check the relative loss of heavy metals between extractions for the Pontiac and Indianapolis sludge ash samples.

TABLE 6. SERIAL EXTRACTION PROCEDURE

REAGENT	TIME	SOLN:SOLID RATIO	REAGENT pH	PURPOSE
0.05 M $CaCl_2$	16 hrs	10:1	6.2	Release easily exchangeable metals
0.1 M $Na_4P_2O_7$	16 hrs.	80:1	10.1	Organically bound metals (for sludges) Al oxides (for ashes)
0.2 M Oxalic Acid/0.4 M NH_4 Oxalate	2 hrs.	variable	3.3	Dissolution of amorphous Fe and Al oxides
0.22 M Sodium Citrate/0.11 M Sodium Bicarbonate + 10 g sodium dithionite (CDB)	2-3 days	100:1	5.6	Dissolution of remaining Fe and Al oxides (mainly crystalline)
HF/Aqua Regia	3-4 hrs.	N/A[a]		Total dissolution

[a] Not Applicable

GENERAL AMES TEST PROCEDURE

Strain Storage -- Five Salmonella typhimurium tester strains used for mutagenic testing were supplied by Dr. Ames via paper discs, each of which had been saturated with a nutrient culture of one of the strains.

A nutrient broth was made up by combining 4 g of Bacto Difco nutrient broth and 2.5 g of sodium chloride with 493.5 mℓ of double-distilled water in a 500 mℓ flask. The nutrient broth was then divided among five 250 mℓ glass screw-capped bottles and autoclaved for 30 minutes at 120°C and 20 lbs. of pressure.

Upon receipt of the tester strains, each of the bacterial discs were lifted from their packets with sterile forceps and dropped into a bottle of nutrient broth. The five bottles were incubated at 37°C overnight ($\leq$ 16 hours) with shaking (100 rpm).

Sixteen hours after inoculating the nutrient broth, 4 mℓ volumes were withdrawn from each with a sterile cotton-plugged 5 mℓ serological pipet and combined with 350 µℓ of dimethyl sulfoxide (DMSO) drawn into a sterile-tipped Finnpipet in a sterile capped borosilicate test tube. This mixture was then divided equally among five 0.5 dram sterile glass screw-capped vials. The 25 total vials were placed upright in a beaker containing 95% ethanol (to ensure even freezing) and lowered into an Ultra-Low Revco freezer at -70°C.

Master Plates -- Master plates for each tester strain were made by scraping the frozen surface of the storage vial with a sterile wooden applicator stick, stirring in 5 mℓ of sterile nutrient broth contained in a 15 x 25 mm sterile test tube which was then incubated overnight with shaking at 37°C. The $\leq$ 16 hour deadline is adhered to because near the end of that time interval the bacteria are growing most actively (still in the log phase of growth) but coming close to entering the following, stationary phase. Within 16 hours, then, a loopful of nutrient broth culture is streaked on the surface of a prepared agar plate.

Sixteen hours after inoculation of the nutrient broth each of the tester strains was streaked onto the plate by dipping a sterile micro loop into the appropriate overnight culture then drawing it across the agar surface. After overnight incubation at 37°C these plates were stored at 3°C for two to three months. After this length of time the agar will begin to dry and new master plates must be made. Details of the preparation of the minimal glucose agar and other reagents used in this portion of the research can be found in McKiernan[82]. Each time a new master plate was made, the bacterial growth was checked to make sure the characteristic genetic markers of the tester strains had not been lost. This included tests for the his^- growth requirement, the rfa or deep rough marker, sensitivity of the strains to ultraviolet light, presence of the R factor, and quantification of the spontaneous reversion rate of each strain. In addition, the strains are tested with positive controls to verify their sensitivity

to induced mutations. The compounds used were sodium azide for strains TA 100 and TA 1535, 9-aminoacridine for TA 1537, and 4-nitrobiphenyl for TA 98 (all without activation). With activation, strain TA 1538 was checked with 2-aminoanthracene and TA 98 were tested with benzo(a)-pyrene.

Sample Testing -- Various extraction samples of the incinerated sludge were tested by the following method.

In preparation for each test session minimal agar plates were poured and labeled; top agar was prepared in 100 mℓ volumes and sterilized, nutrient broth was made up, dispensed in 5 mℓ aliquots into 16 x 25 mm capped test tubes, sterilized and refrigerated; Finnpipet tips were arranged in 250 mℓ beakers, covered with gauze and aluminum foil and sterilized; packaged paper towels were separated, stacked in piles of ten, wrapped in brown paper and sterilized; and a 0.5 mM biotin/0.5 mM histidine solution was prepared and stored at -18°C. The sterile solution was then pipetted in 10 mℓ volumes into sterile 16 x 25 mm test tubes, covered with paraffin and frozen.

The day before the test, five nutrient broth tubes were removed from the refrigerator and warmed to room temperature. Each was then inoculated with a flamed micro loop that had picked up bacteria from the master plate. The nutrient broth tubes were then incubated at 37°C with shaking of 100 rpm.

Approximately 14 hours later, the water bath was heated to 45°C and racks of sterile 16 mm x 125 mm capped test tubes were placed inside. Top agar was melted by suspending the bottle(s) in a beaker of boiling water over a hot plate then cooled to 45°C in the water bath. For each 100 mℓ bottle of agar to be used one tube of 0.5 mM histidine/biotin was removed from the freezer and thawed, then added to the molten agar. A Cornwall Continuous Pipetting outfit equiped with a 5 mℓ syringe adjusted to dispense 2 mℓ volumes had been sterilized separately and was reassembled with sterile forceps. With this pipetting device, 2 mℓ of top agar containing a trace amount of histidine and biotin was deposited in each of the warmed test tubes. One sterile Finntip for each sample was then inserted between the folds of a sterile paper towel (three to four tips per towel).

At this time, the five nutrient broth cultures were removed from the incubator and their caps replaced by paraffin. Testing began for one strain at a time by inserting into the culture tube a sterile 1 mℓ plugged pipet (in 0.01 mℓ divisions) equipped with a Gelasfirn-pi-pump pipetting aid. The tube was sealed with paraffin to prevent contamination. Volumes of 0.1 mℓ were then drawn up and released into the agar tubes. A Finnpipet was then used to quickly add 0.1 mℓ of a sterile sample, with the appropriate sterile pipet tip, to the tube. The agar mixture was then vortexed immediately and poured over a minimal agar plate, swirling for even distribution across the surface.

Spontaneous reversion control plates contained only the top agar and 0.1 mℓ of the tester strain and were poured in triplicate. Negative

control plates tested the solvent used in the sample and were tested in duplicate. Sample plates were poured in duplicate initially, and those demonstrating elevated levels of induced reversions were retested in triplicate one or more times.

When the samples were tested with enzyme activation, 310 µℓ of the activation mix (S-9) were added to the top agar just after the sample, and additional negative control test plates were poured to check the sterility of the mix.

Upon completion of the test session all plates were packed in a cardboard box lined and covered with paper towel. A tray was then placed over the top to ensure darkness, and the box was lowered into the incubator for 48 hours at 37°C. After this time they were removed and scored for reversions using a Darkfield Quebec American Colony Counter.

Extractions Tested According to the Ames Method -- Information concerning the extraction tested is presented in Table 7. Details concerning the preparation of these extracts are given in McKiernan[82].

Extract Dilutions -- Extract dilutions were made by transferring with a sterile pipet or Finntip a certain volume of the full-strength sample to a sterile, capped test tube containing an appropriate amount of the same solvent. (For example to make a 1:100 dilution of an acetone extraction redissolved in DMSO, 50 µℓ of the DMSO extract is added to 5 mℓ of DMSO).

In tabulating results, revertants were taken from the linear portion of a dose-response curve and were reported at the highest concentration which was not toxic to the bacteria. To determine toxicity, the plates were checked for the presence of a lawn of growth which indicates strain survival of the test compound. If this confluent growth is absent, or revertant levels on the test plate fall below spontaneous reversions on the control plate, the sample is considered toxic to the bacteria at that concentration and must be diluted for further testing.

Testing of several controls with varying pHs provided evidence of bacterial survival over the range of pH 2 to pH 11. In instances where toxicity was suspected, test plates were scored for revertants after 48 hours of incubation as directed, then reincubated for an additional 24 hours and scored again. This follows the suggestion of de Serres and Shelby[68]. and is based on the observation that toxic compounds may delay growth of revertants.

To provide an example of toxicity, dilution and generation of a dose-response curve, tests of the acetone extraction of 50 g of Jersey City sludge are discussed here. The extract resuspended in DMSO was not completely dry due to the presence of an oily residue; therefore, weights of the extract to determine the volume of DMSO addition to yield a 20 - 40 mg/mℓ concentration could only be estimated. As a result, it is likely that the final sample was more concentrated than this suggested level as testing indicated high toxicity to the Salmonella typhimurium. Dilution from 1/10

TABLE 7. EXTRACTION PROCEDURES FOR AMES TEST

EXTRACTION TYPE	REAGENT	SOLUTION (ml) / SOLID (g) RATIO	CONTACT TIME	COMMENTS
Inorganic (ASTM Method A, Neutral)	Double-Distilled Water	4:1	48 hours	Collects Water-Soluble Materials from the Ash.
Inorganic (Acidic)	1.0 N Nitric Acid	5:1	24 hours	Collects Strong Acid-Soluble Materials.
Inorganic (Acidic)	0.1 M Hydroxylamine Hydrochloride in 0.01 M HNO_3	50:1	30 min.	Dissolves Mn Oxides.
Inorganic (Near-neutral)	0.05 M Calcium Chloride	10:1	16 hours	Releases Easily Exchangeable Species.
Inorganic (Basic)	0.01 M Sodium Hydroxide	10:1	16 hours	Dissolves Al oxides.
Inorganic (Basic)	0.1 M Sodium Pyrophosphate	10:1	16 hours	Dissolves Al oxides.
Inorganic (Acidic)	0.2 M Oxalic Acid/ 0.4 M Ammonium Oxalate	13:1	1 hour	Dissolves Oxides of Fe and Al.
Inorganic (IAEA Method, Neutral)	Double-Distilled Water	12:1	1 week	Collects Water-Soluble Materials.
Organic (Acidic)	0.5 N Acecic Acid	5:1	24 hours	Collects Weak Acid-Soluble Organics.
Organic (Strong Solvent)	Dimethyl Sulfoxide	10:1	24 hours and 1 week	Collects Organics.
Organic (Soxhlet)	95% Ethanol	20:1	12 hours	Collects Organics.
Organic (Soxhlet)	Benzene	20:1	12 hours	Collects Organics.
Organic	Acetone	4:1	1 week	Collects Organics.
Inorganic/ Organic (Resin Column)	Water/ Eluted With Acetone	4:1	48 hours	Collects and Concentrates Organics.

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to 1/10,000 were carried out and the sample retested. The dose-response curve determined for this extraction with strain TA 98 in the absence of S-9 is presented in Figure 1. Results in this case were reported for the 1:1000 dilution.

Ratios of diluted to original samples ranged from 1:10 to 1:10,000

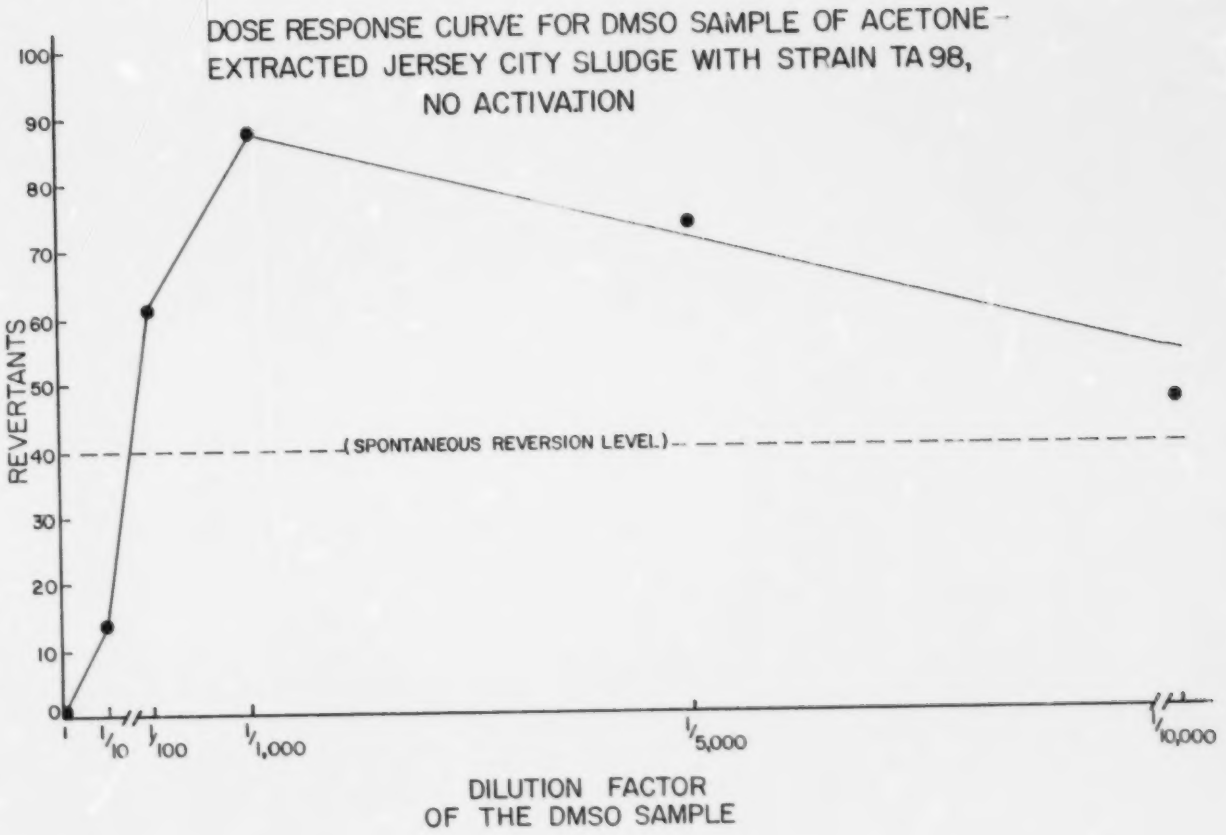

Figure 1. Dose Response Curve for DMSO Sample of Acetone-Extracted Jersey City Sludge with Strain TA 98, No Activation

SECTION 6

RESULTS AND DISCUSSION

MICROSCOPY STUDIES

Both optical and electron photomicrographs were taken during this study. Optical microscopy of selected samples generally revealed particles of an irregular sintered appearance, although a spherical morphology was occasionally observed. The color ranged from reddish-brown to black, with the dominant pigmentation due to oxides of iron.

Electron microscopy revealed to a greater degree various substructures in the ash samples. Both elongated and spherical particles were found although, at the magnification used, crystalline structures were not visible. A greater degree of regularity among particles from fluidized bed incinerators was clearly evident. Fibrous structures of organic origin were found in the sample from Jersey City, New Jersey. These structures disappeared upon volatilization at 550°C. Although all stations reported temperatures between 730 and 925°C the variety and composition of particles found suggest that both "hot" and "cold" spots exist in these incinerators. The temperatures would be expected to volatilize virtually all organic matter but are not high enough to cause melting (and a resultant spherical shape) of the minerals which have been identified in sludge ashes.

A few representative XRF scans are shown in Figure 2. No attempt was made to obtain quantitative information by this method, thus the relative peak heights of Figure 2 provide only qualitative data. Perhaps the most obvious feature of these scans is that they reveal not only the major elements but also those elements added during the course of the treatment process. Thus Detroit adds waste pickle liquor, Port Huron adds alum, and Pontiac adds both ferric chloride and lime.

CHEMICAL CHARACTERISTICS AND COMPOSITION

Pertinent information and basic data characterizing the sludge ash samples are presented in Table 8. Similar data for the corresponding dewatered sludge samples are found in Table 9. Both pH and volatile content are within the range reported previously, being less than one percent for each sludge ash sample except for samples from Jersey City and Port Huron. The sample from Jersey City, as indicated, was found to contain fibrous materials which is organic in nature.

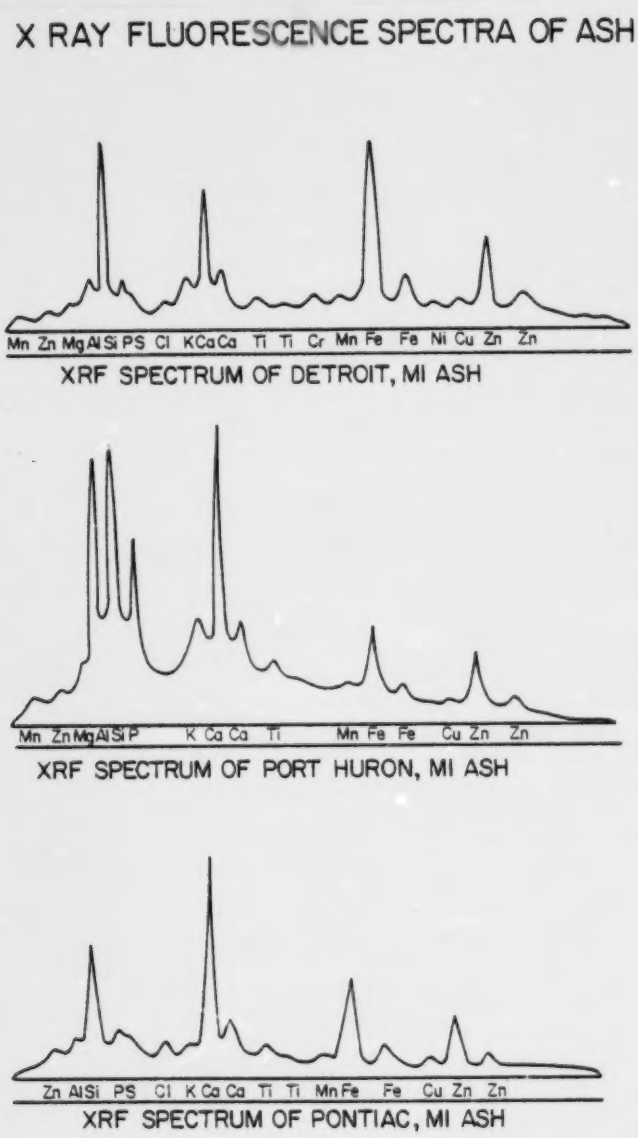
X RAY FLUORESCENCE SPECTRA OF ASH
Mn Zn Mg Al Si P S Cl K Ca Ca Ti Ti Cr Mn Fe Fe Ni Cu Zn Zn
XRF SPECTRUM OF DETROIT, MI ASH
Mn Zn Mg Al Si P K Ca Ca Ti Mn Fe Fe Cu Zn Zn
XRF SPECTRUM OF PORT HURON, MI ASH
Zn Al Si P S Cl K Ca Ca Ti Ti Mn Fe Fe Cu Zn Zn
XRF SPECTRUM OF PONTIAC, MI ASH

Figure 2. X Ray fluorescence spectra of ash.

TABLE 8. GENERAL CHARACTERIZATION FOR SLUDGE ASH SAMPLES

LOCATION	TYPE OF INCINERATOR	HIGHEST OPERATING TEMPERATURE (°C)	DISPOSAL METHOD	PHYSICAL CHARACTERISTICS	pH	% VOLATILE MATTER	CHEMICAL ADDITION	PURPOSE OF CHEMICAL ADDITION	Fe(III) & Al ADDED - mg as $FeCl_3$/g dry sludge *
DETROIT	Multiple Hearth	925	Landfill	Brown Variable	7.71	0.28	Polymer, Pickle Liquor	Sludge Conditioning, Phosphorus Removal	
GREEN BAY	Multiple Hearth	760	Landfill	Light Brown Very Fine	8.81	0.59	$FeCl_3$ Alum	Phosphorus Removal	93.3
INDEPENDENCE	Fluidized Bed	760	Lagoon to Landfill	Gray Very Fine	7.94	0.89	Polymer	Sludge Conditioning	
INDIANAPOLIS	Multiple Hearth	980	Lagoon to Landfill	Brown Fine	8.15	0.20	Polymer	Sludge Conditioning	
JERSEY CITY	Multiple Hearth	840	Landfill	Brownish-Gray intermixed with fibrous material	9.81	5.85	$FeCl_3$, Lime	Sludge Conditioning	158.6
MONTROSE	Multiple Hearth	870	Lagoon	Dark Brown Very Fine	12.20	0.49	$FeCl_3$, Lime, Polymer	Sludge Conditioning, Phosphorus Removal	97.5
PARSIPPANY	Multiple Hearth	925	Lagoon	Reddish-Brown Variable	8.79	0.04	Polymer	Sludge Conditioning	
PONTIAC	Multiple Hearth	760	Landfill, Lagoon occasionally	Brown Fine	11.72	0.16	$FeCl_3$ Lime Polymer	Sludge Conditioning, Phosphorus Removal	240.0
PORT HURON	Fluidized Bed	730	Landfill	Black and Brown Fine	8.02	1.35	Alum, Polymer	Sludge Conditioning, Phosphorus Removal	46.2
WALDWICK	Fluidized Bed	790	Landfill	Light Brown Fine	5.89	0.53	Polymer	Sludge Conditioning	

*Chemical dosage normalized to a weight per weight basis of dried sludge solids, regardless of the manner or point of addition in the treatment process.

TABLE 9. GENERAL CHARACTERISTICS OF DEWATERED SLUDGE SAMPLES

LOCATION	FLOW TO TREATMENT PLANT (MGD)	DRY SLUDGE PRODUCED (METRIC TONS PER DAY)	COLOR	% SOLIDS		% VOLATILE CONTENT		pH
				LAB VALUE	AVERAGE REPORTED BY CITY	LAB VALUE	AVERAGE REPORTED BY CITY	
DETROIT	615	435	Black	18.2	21.4	54.6	55 - 60	4.95
GREEN BAY	36	24	Dark Brown	37.8	32 - 38	68.4	65 - 70	5.43
INDEPENDENCE	6	6.1	Dark Brown	35.1	30 - 35	60.0	65 - 75	5.73
INDIANAPOLIS	163	136	Dark Brown	33.1	28.3	30.1	37*	5.79
JERSEY CITY	53	27	Gray	24.1	26.0	62.5	55 - 60	7.17
MONTROSE	18	18	Black	21.1	23.0	43.2	45 - 50	6.53
PARSIPPANY	8	4.4	Black	15.8	18 - 22	69.1	65 - 70	5.38
PONTIAC	18	16	Dark Brown	29.2	20 - 25	30.8	30 - 35**	7.23
PORT HURON	9	9.3	Black	16.0	18.2	52.2	55 - 60	5.91
WALDWICK	5	3.2	Black	17.0	17 - 18	75.6	75	5.24

*At Indianapolis, a portion of the ash discharged from the incinerator is recycled for use as a dewatering aid. This is why the volatile content of the sludge averages 37%.

**At Pontiac, the sludge undergoes anaerobic digestion. This accounts for the lower volatile content.

Each sludge ash sample, except for Waldwick, produced an alkaline reaction when combined with double distilled water, as might be expected from the dissolution of surface lime deposits. It should also be noted that the three locations, Jersey City, Montrose, and Pontiac, which add lime at the treatment facility also display the highest pH values. It is possible that the pH is also related to the amount of amorphous iron oxides because of the release of protons during hydrolysis reactions. The pH of the dewatered sludge is, without exception, lower than that of the corresponding sludge ash. This is to be expected since acidic volatiles, such as H_2S and CO_2, are driven off during incineration.

Values for bulk elemental analysis of each of the ten ashes are presented in Table 10 with corresponding data for the dewatered sludges in Table 11. The ashes are composed chiefly of aluminum, calcium, iron, sodium, and of course silicon and oxygen. Elevated values of iron, aluminum, and calcium are evident in those locations where ferric chloride, aluminum sulfate, or lime are added for phosphorus removal or sludge conditioning. In both Tables 10 and 11 the effect of various chemical additions can be clearly seen as in the case of iron for Detroit, Jersey City, Montrose, and Pontiac; aluminum for Port Huron, and lime for Montrose and Pontiac. Selenium was below the detection limit for each sample.

On a fixed solids basis the concentration of the various metals in the dewatered sludge samples generally corresponds to the concentration in the respective ashes.

ASTM METHOD A - WATER SHAKE EXTRACTION RESULTS

This extraction method utilized double distilled water as the leaching agent. The pH was not controlled but was buffered by the sludge ash material being tested close to the equilibrium pH values for each as were presented in Table 8. Results for this test are given in Table 12. For purposes of comparison, bulk concentrations (μg/g), amount extracted normalized to the amount of ash present (μg/g), aqueous concentration of the extractant (mg/ℓ), and percent extraction are included.

Table 12 reveals that the alkaline pH under which the extractions took place results in low amounts of leachable metals. Both solubility and surface sorption controls act to minimize the release of metals under those conditions. In several cases the amount extracted was below the detection limit for the metal under investigation. From this data it is difficult to make any generalizations regarding parameters, other than pH, which might increase or decrease leachability.

U.S. EPA EXTRACTION PROCEDURE (EP) RESULTS

Unlike the ASTM Method A test the pH was controlled throughout this procedure at 5.0 ± 0.2 with a 0.5 N acetic acid as outlined in Section 5.

TABLE 10. BULK CONCENTRATION OF METALS IN SLUDGE ASH (µg/g Dry Wt.)

LOCATION	Al	As	Ca	Cd	Cr	Cu	Fe	Hg	K	Mn	Na	Ni	Pb	Se	Zn
DETROIT	34800	3.7	74000	270	6560	4700	305000	8.4	6600	2000	10900	3900	1190	< 4.0	10700
GREEN BAY	35600	1.2	146000	21	2200	3200	82000	8.8	6600	5500	24600	840	760	< 4.0	4400
INDEPENDENCE	64000	1.7	68000	900	810	3300	50000	3.1	8800	2500	25800	880	1070	< 4.0	6000
INDIANAPOLIS	75000	3.7	102500	190	4800	6000	62000	5.0	6500	1000	19400	1230	2080	< 4.0	15100
JERSEY CITY	38000	8.1	118000	9	1670	4900	101000	7.0	3800	800	29900	1200	1620	< 4.0	9000
MONTROSE	9200	20.0	380000	4	350	1500	171700	4.5	800	500	16100	690	90	< 4.0	900
PARSIPPANY	38000	2.7	90000	7	2060	5500	82500	9.2	7400	5000	28000	670	230	< 4.0	6400
PONTIAC	29200	20.8	245500	5	1150	3600	179000	9.0	2700	4300	8700	1700	1650	< 4.0	8000
PORT HURON	108000	1.3	58500	14	870	7000	41300	5.8	23000	1800	265000	980	1600	< 4.0	23800
WALDWICK	22800	1.8	80000	45	350	4500	40700	2.0	3900	5000	13500	270	910	< 4.0	4000

TABLE 11. BULK CONCENTRATION OF METALS IN DEWATERED SLUDGE SAMPLES (μg/g DRY WT.)

LOCATION	As	Cd	Cr	Fe	Ni	Pb	Se
DETROIT	0.5	120	2230	168,500	950	430	<4.0
GREEN BAY	0.4	7	820	21,200	140	240	<4.0
INDEPENDENCE	0.5	250	440	19,200	190	480	<4.0
INDIANAPOLIS	0.8	90	3500	47,300	840	1040	<4.0
JERSEY CITY	1.6	4	340	44,000	230	530	<4.0
MONTROSE	4.1	1	80	78,900	130	20	<4.0
PARSIPPANY	0.5	3	420	21,200	140	70	<4.0
PONTIAC	2.0	2	560	131,000	930	1090	<4.0
PORT HURON	0.4	5	380	21,100	210	420	<4.0
WALDWICK	0.4	18	90	9500	80	240	<4.0

TABLE 12. ASTM METHOD A - WATER SHAKE EXTRACTION RESULTS FOR SLUDGE ASH SAMPLES

	As				Cd				Cr			
	BULK	EXTRACTION			BULK	EXTRACTION			BULK	EXTRACTION		
LOCATION	µg/g	µg/g	mg/ℓ	% EXT.	µg/g	µg/g	mg/ℓ	% EXT.	µg/g	µg/g	mg/ℓ	% EXT.
DETROIT	3.7	0.020	0.002	0.5	270	0.013	0.001	— [a]	6560	0.400	0.040	— [a]
GREEN BAY	1.2	<0.010		a	21	0.002	0.002	— [a]	2200	0.052	0.005	— [a]
INDEPENDENCE	1.7	<0.010		a	900	0.025	0.003	— [a]	810	0.030	0.003	— [a]
INDIANAPOLIS	3.7	0.080	0.008	2.2	190	0.010	0.001	— [a]	4800	0.111	0.011	— [a]
JERSEY CITY	8.1	0.010	0.001	0.1	9	<0.001		a	1670	0.400	0.040	0.02
MONTROSE	20.0	<0.010		a	4	<0.001		a	350	0.074	0.007	0.02
PARSIPPANY	2.7	<0.010		a	7	<0.001			2060	0.200	0.020	— [a]
PONTIAC	20.8	<0.010		a	5	0.001	0.0001	0.02	1150	0.350	0.035	0.03
PORT HURON	1.3	<0.010		a	14	0.008	0.001	0.06	870	0.067	0.007	— [a]
WALDWICK	1.8	<0.010		a	45	0.008	0.001	0.02	350	0.018	0.002	— [a]

[a] percent extracted is less than 0.01.

TABLE 12 continued

LOCATION	Ni				Pb				Se			
	BULK	EXTRACTION			BULK	EXTRACTION			BULK	EXTRACTION		
	µg/g	µg/g	mg/ℓ	% EXT.	µg/g	µg/g	mg/ℓ	% EXT.	µg/g	µg/g	mg/ℓ	% EXT.
DETROIT	3900	0.450	0.045	0.01	1190	<0.008		a	<4.0	<0.020		a
GREEN BAY	840	0.050	0.005	a	760	0.023	0.002	— a	<4.0	<0.020		a
INDEPENDENCE	880	0.750	0.075	0.09	1070	0.098	0.010	— a	<4.0	<0.020		a
INDIANAPOLIS	1230	1.000	0.100	0.08	2080	0.053	0.005	— a	<4.0	<0.020		a
JERSEY CITY	1200	0.150	0.015	0.01	1620	<0.008		a	<4.0	<0.020		a
MONTROSE	690	0.100	0.010	0.01	90	0.038	0.004	0.04	<4.0	<0.020		a
PARSIPPANY	670	0.150	0.015	0.02	230	0.008	0.001	— a	<4.0	<0.020		a
PONTIAC	1700	0.250	0.025	0.01	1650	0.008	0.001	— a	<4.0	<0.020		a
PORT HURON	980	0.100	0.010	0.01	1600	0.023	0.002	— a	<4.0	<0.020		a
WALDWICK	270	1.100	0.110	0.4	910	<0.008			<4.0	<0.020		a

[a]percent extracted is less than 0.01.

Results for this test conducted on the sludge ash and dewatered sludge are given in Tables 13 through 17. Comparing the results with those obtained from the ASTM test indicates that the leachability of trace metals is strongly dependent on pH. For example, as much as 85.6% of the total nickel was extracted from the Green Bay ash sample during the EP extraction.

For all elements studied in this test, their leachability appears to be strongly dependent on the prior addition of iron (III) and/or aluminum salts to the wastewater or sludge. Oxyhydroxides of both iron and aluminum, which would be expected to form in response to the addition of these salts, exert surface sorption and solubility controls on trace elements which are pH dependent. Cities which add iron (III) or aluminum salts include Green Bay, Jersey City, Montrose, Pontiac, and Port Huron. Detroit adds ferrous pickle liquor for its phosphorus removal, however it is not clear what proportion of the final pickle liquor product exists as iron (III) in the aeration tanks. It appears from the relatively low percentages of trace metals extracted that the iron pickle liquor does not behave towards trace metals in the same way as iron (III) salts do. Singer and Theis[83] point out that ferrous iron added to activated sludge aeration tanks is not rapidly oxidized to ferric iron.

In the EPA extraction test, the correlation with iron (III) and aluminum addition and the amount of metal cations released is a direct one. For arsenic which has an anionic aqueous chemistry, an inverse relationship exists. At pH 5, the most common forms of iron and aluminum oxides are positively charged implying that the proton can successfuly compete for surface sites against trace metal cations. Under these conditions, however, arsenic (existing most probably as arsenate or arsenite) will be electrostatically attracted to the surface. Attractive chemical forces may also exist. These trends are summarized in Table 18 using average figures from Tables 13 through 17. An additional feature of the data is that, with the exception of Cd, incineration acts to increase the leachability of cationic elements if Fe (III)/Al (III) salts have been added. For As and Cd, incineration decreases their leachability. If these salts are not added, cationic elements are generally less leachable in the ash than the sludge.

It should be noted that there is little correlation between amount of an element released and bulk levels of that element or Fe and Al in the ash and sludge samples. For example, Detroit ash contains 3.7 times as much iron on a bulk basis as the Green Bay ash and about the same amount of aluminum, yet much higher concentrations of cationic elements were extracted from the Green Bay sample. The pH of the Detroit and Green Bay samples are approximately the same. Pontiac contains almost 2.2 times as much iron as Parsippany but nearly the same amount of aluminum. However lower concentrations of lead and nickel, and the same amount of cadmium were extracted from the Pontiac ash sample.

Federal guidelines suggest that if heavy metal concentrations generated by the EPA Extraction Procedure are greater than or equal to 100 times the

TABLE 13. EPA EXTRACTION PROCEDURE: RESULTS FOR ARSENIC

	ASH				DEWATERED SLUDGE			
	BULK	SOLUBLE			BULK	SOLUBLE		
LOCATION	ug/g	ug/g	mg/ℓ	% ext.	ug/g	ug/g	mg/ℓ	% ext.
Detroit	3.66	0.64	.03	17.5	0.51	.08	.004	15.7
Green Bay	1.21	.02	.001	1.7	0.40	.04	.002	10.0
Independence	1.72	.25	.01	14.5	0.50	.02	.001	4.0
Indianapolis	3.70	.50	.03	13.5	0.80	.16	.008	20.0
Jersey City	8.05	.13	.01	1.6	1.55	.08	.004	5.2
Montrose	20.0	.45	.02	2.3	4.08	.02	.001	.5
Parsippany	2.66	.40	.02	15.0	0.51	.05	.003	9.8
Pontiac	20.78	.81	.04	3.9	2.04	.03	.002	1.5
Port Huron	1.33	.02	.001	1.5	0.40	.02	.001	5.0
Waldwick	1.79	.05	.003	2.8	0.40	.03	.002	7.5

TABLE 14. EPA EXTRACTION PROCEDURE: RESULTS FOR CADMIUM

LOCATION	ASH				DEWATERED SLUDGE			
	BULK	SOLUBLE			BULK	SOLUBLE		
	ug/g	ug/g	mg/ℓ	% ext.	ug/g	ug/g	mg/ℓ	% ext.
Detroit	270	1.18	.06	.4	120	4.68	.23	3.9
Green Bay	21	.51	.03	2.4	7	0.33	.02	4.7
Independence	900	8.92	.45	1.0	250	4.40	.22	1.8
Indianapolis	190	2.18	.11	1.1	90	1.89	.09	2.1
Jersey City	9	1.24	.06	13.8	4	1.35	.07	33.8
Montrose	4	.70	.04	17.5	1	0.51	.03	51.0
Parsippany	7	.35	.02	5.0	3	0.20	.01	6.7
Pontiac	5	.25	.01	5.0	2	0.53	.03	26.5
Port Huron	14	.35	.02	2.5	5	0.32	.02	6.4
Waldwick	45	.28	.01	0.6	18	0.38	.02	2.1

TABLE 15. EPA EXTRACTION PROCEDURE: RESULTS FOR CHROMIUM

LOCATION	ASH				DEWATERED SLUDGE			
	BULK	SOLUBLE			BULK	SOLUBLE		
	ug/g	ug/g	mg/ℓ	% ext.	ug/g	ug/g	mg/ℓ	% ext.
Detroit	6600	1.18	0.06	0.1	1900	22.55	1.13	1.2
Green Bay	2200	9.08	0.45	.4	820	0.38	.02	.05
Independence	800	1.18	0.06	.1	240	1.84	.09	.8
Indianapolis	4800	.67	0.03	.01	1800	2.84	0.14	.2
Jersey City	1700	15.10	0.76	.9	340	1.08	0.05	.3
Montrose	400	25.50	1.28	6.4	80	1.53	.08	1.9
Parsippany	2100	0.67	0.03	.03	420	0.28	0.01	.1
Pontiac	1200	15.85	0.79	1.3	250	6.99	.35	2.8
Port Huron	870	0.55	0.03	.1	380	0.97	.05	.3
Waldwick	350	0.04	0.002	.01	90	0.06	.003	.1

TABLE 16. EPA EXTRACTION PROCEDURE: RESULTS FOR NICKEL

LOCATION	ASH				DEWATERED SLUDGE			
	BULK	SOLUBLE			BULK	SOLUBLE		
	ug/g	ug/g	mg/ℓ	% ext.	ug/g	ug/g	mg/ℓ	% ext.
Detroit	3900	14.14	0.71	0.4	950	130.55	6.53	13.7
Green Bay	840	719.05	35.95	85.6	140	21.09	1.05	15.1
Independence	880	7.30	0.37	0.8	190	6.95	0.35	3.7
Indianapolis	1230	3.30	0.16	0.3	800	218.63	10.93	27.3
Jersey City	1200	948.01	47.40	79.0	230	179.63	8.98	78.1
Montrose	690	408.00	20.40	59.1	130	39.03	1.95	30.0
Parsippany	670	345.20	17.26	51.5	100	15.45	0.77	15.5
Pontiac	1700	205.02	10.25	12.1	900	404.49	20.22	44.9
Port Huron	980	786.06	39.30	80.2	200	108.03	5.40	54.0
Waldwick	270	4.20	0.21	1.6	80	41.28	2.06	51.6

TABLE 17. EPA EXTRACTION PROCEDURE: RESULTS FOR LEAD

LOCATION	ASH				DEWATERED SLUDGE			
	BULK	SOLUBLE			BULK	SOLUBLE		
	ug/g	ug/g	mg/ℓ	% ext.	ug/g	ug/g	mg/ℓ	% ext.
Detroit	1200	3.13	.16	.3	430	1.65	.08	.4
Green Bay	800	1.68	.08	.2	200	1.22	.06	.6
Independence	1100	1.80	.09	.2	480	.89	.05	.2
Indianapolis	2100	3.61	.18	.2	1000	.39	.02	.04
Jersey City	1600	2.95	.15	.2	500	.20	.01	.04
Montrose	90	4.06	.20	4.5	20	.14	.01	.7
Parsippany	230	1.90	.10	0.8	70	.67	.03	1.0
Pontiac	1700	1.88	.09	.1	470	.30	.02	.1
Port Huron	1600	1.47	.07	.1	420	.65	.03	.2
Waldwick	900	1.30	.07	.1	240	.61	.03	.3

TABLE 18. AVERAGE PERCENT REMOVED IN EPA-EP FOR ASH AND SLUDGE

ELEMENT	ASH		SLUDGE	
	Fe(III)/Al ADDED	Fe(III)/Al NOT ADDED	Fe(III)/Al ADDED	Fe(III)/Al NOT ADDED
As	2.2	12.6	4.4	11.5
Cd	8.2	1.6	24.5	3.3
Cr	2.0	0.03	0.8	0.3
Ni	63.2	10.9	43.6	21.2
Pb	1.0	0.3	0.3	0.4

Drinking Water Standards the water is classified as hazardous. Based on this criterion, none of the ashes analyzed are hazardous according to their leachate concentrations.

INTERNATIONAL ATOMIC ENERGY AGENCY (IAEA) LEACH TEST RESULTS.

This extraction test is a batch equilibration of sludge ash in double distilled water which proceeds serially. The leachate was decanted each day for the first week and every seventh day thereafter for 21 weeks. A summary of the results is presented in Table 19. This test was performed on six ash samples only.

A trend toward greater metal release for those ashes which were obtained from treatment facilities which use iron or aluminum salts (Jersey City, Pontiac, Port Huron) is apparent from the data. Again, there is an inverse relationship for arsenic which has an anionic aqueous chemistry. The effect of pH can also be seen especially for the Pontiac ash which has an equilibrium pH of 11.7. For example the Pontiac sample had more ferric chloride added than Jersey City, however the release of cationic metals is less than for Jersey City except for lead.

The amount of a given metal released was found to change as the test proceeded, revealing some interesting trends. Figures 3, 4, and 5 indicate the change in cumulative metal concentration for a series of equilibration periods for three ash samples. Figure 6 shows the IAEA release data for Detroit in greater detail. Here changes in concentration for the indicated metals are shown along with pH. pH rises sharply during the test, most probably due to the exposure to solution of base deposits. While the metal cations Cr and Cd show a gradual declining trend, As released rises simultaneously with pH. As suggested previously such behavior would be expected as the surface assumes a negative charge at the high pH. Arsenic is poorly held under these conditions. Trends similar to those of Figure 6 were also found for other ash samples. Such changes during the IAEA test suggest a rather non-uniform distribution of major and minor elements both on the surface and as a function of depth in the ash.

The IAEA test does not reflect a kinetic batch experiment wherein the metal concentration in the leachate is measured as a function of time, but rather a batch re-equilibration where the metal concentration is measured in each periodic extraction. This procedure is meant to simulate a landfill disposal environment in that the ash is contacted with a fresh volume of water at different points in time.

SERIAL EXTRACTION RESULTS

The precise mineralogy of trace elements cannot normally be directly measured. Heavy metals are most likely associated with the major minerals of the ash as a mixed mineral or a surface sorbed species. The serial extraction procedure conducted in this study was designed to provide information about heavy metal associations with specific extractable phases.

TABLE 19. IAEA LEACH TEST RESULTS FOR SLUDGE ASH SAMPLES (Total Amount Extracted)

LOCATION	As			Cd			Cr			Ni			Pb		
	BULK µg/g	EXT. µg/g	% EXT.	BULK µg/g	EXT. µg/g	% EXT.	BULK µg/g	EXT. µg/g	% EXT.	BULK µg/g	EXT. µg/g	% EXT.	BULK µg/g	EXT. µg/g	% EXT.
DETROIT	3.7	2.229	60.2	270	0.219	0.08	6560	0.775	0.01	3900	0.470	0.01	1190	0.081	-
INDEPENDENCE	1.7	0.918	54.0	900	0.102	0.01	810	0.227	0.03	880	<.045		1070	0.198	0.02
INDIANAPOLIS	3.7	0.924	25.0	190	0.085	0.04	4800	0.196	-[a]	1230	<.045		2080	0.513	0.02
JERSEY CITY	[illegible].1	0.408	5.0	9	0.067	0.7	1670	2.589	0.2	1200	0.310	0.03	1620	0.342	0.02
PONTIAC	20.8	0.576	2.8	5	0.011	0.2	1150	1.522	0.1	1700	<.045		1650	0.396	0.02
PORT HURON	1.3	0.228	17.5	14	0.118	0.8	870	0.567	0.07	980	0.335	0.03	1600	0.297	0.02

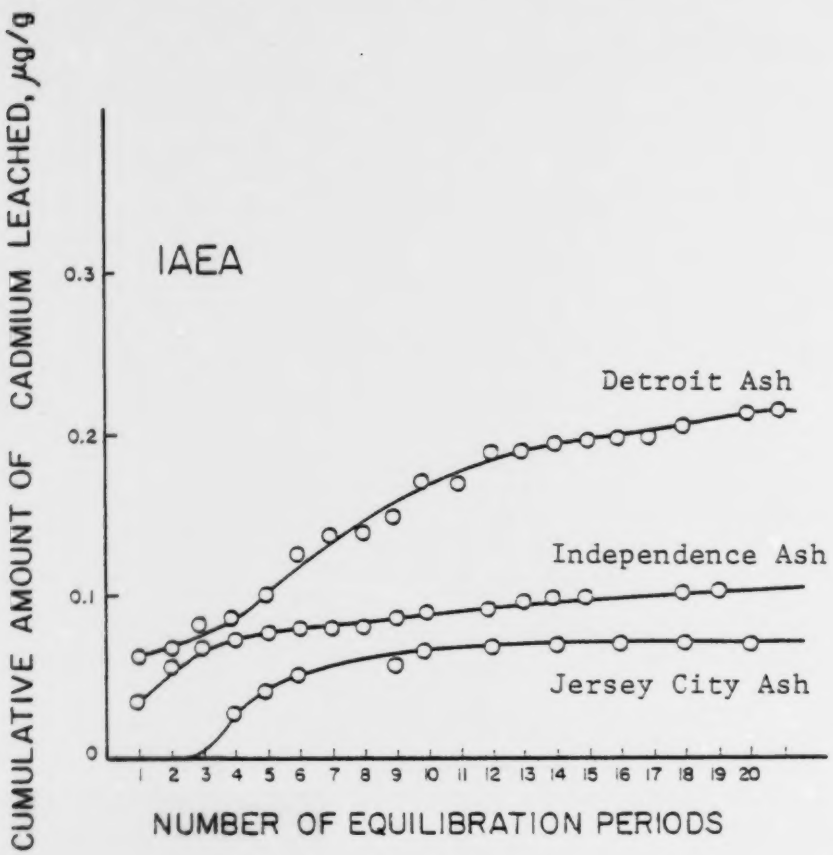

Figure 3. IAEA Leach Test - cadmium concentration in ash leachate.

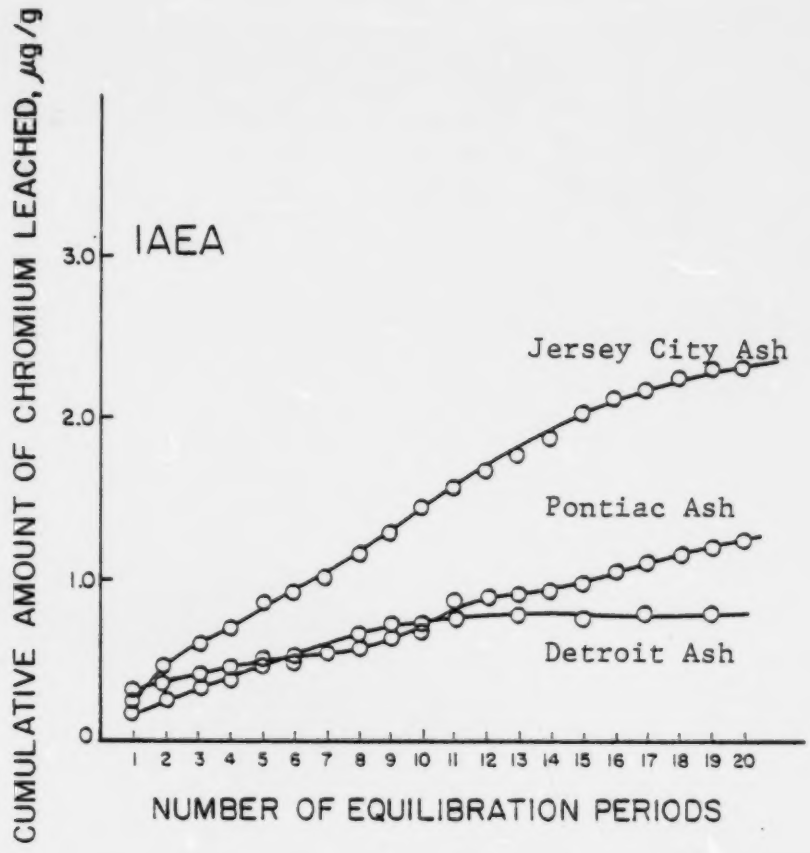

Figure 4. IAEA Leach Test - chromium concentration in ash leachate.

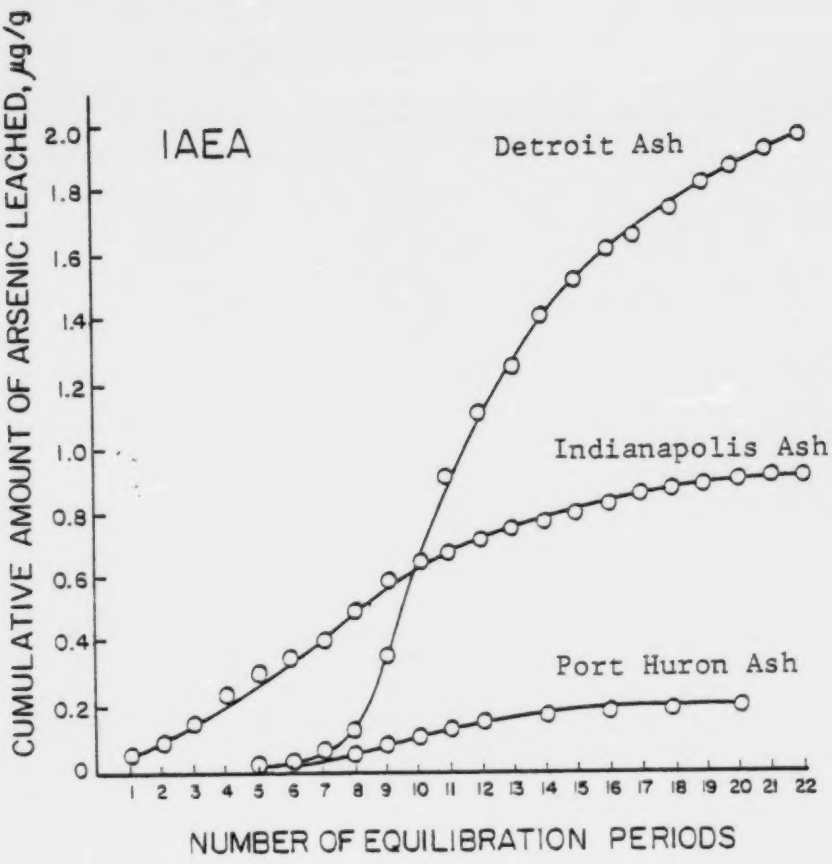

Figure 5. IAEA Leach Test - arsenic concentration in ash leachate.

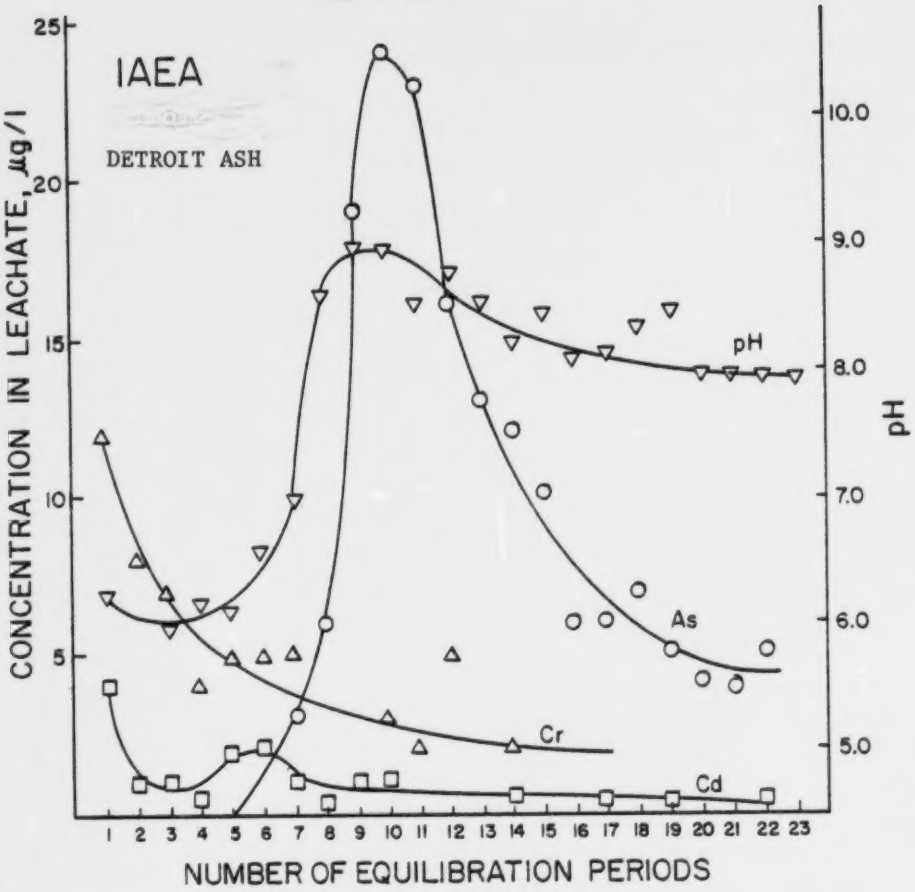

Figure 6. IAEA Leach Test for Detroit sludge ash.

Several of the ashes and two dewatered sludge samples from Indianapolis and Pontiac were subjected to the serial extraction procedure. Even though only two dewatered sludge samples were used some general observations can be made regarding the effect of incineration upon metal associations.

Naturally, the major difference between the composition of the dewatered sludges and the ashes is the organic fraction. As Figures 7 and 8 indicate, the largest percentages of aluminum, cadmium, and lead were extracted from the organic fraction or pyrophosphate extractable phase of the dewatered sludges from Indianapolis and Pontiac. Before incineration the largest percentages of aluminum, cadmium, and lead were extracted from the organic fraction or pyrophosphate extractable phase of the dewatered sludges from Indianapolis and Pontiac. After incineration the largest percentages of aluminum, cadmium, and lead were found in the oxalate or amorphous oxide fraction of the ash. This experiment in itself does not prove that cadmium and lead are bound specifically to amorphous aluminum oxides, however both Cd and Pb have been shown to exhibit a strong attraction to oxides of Al in laboratory aqueous solutions[84,85].

COMPARISONS AMONG SINGLE-REAGENT LEACHATE TESTS

Comparisons among the single-reagent leachate tests that were made are inevitable in a study of this nature. There are, as indicated, certain differences as well as similarities in the way the tests are performed, however in viewing the results of the tests, it is the differences that stand out. Table 20 shows the average <u>total</u> amounts of each trace element released from all ashes for the three tests. Values are normalized so that the ASTM-A test is unity. Clearly this test, with one exception, resulted in the least amount of elements removed. In comparison the IAEA test, which utilized the same reagent, brought about greater amounts of metal cations removed (except for Ni) and much greater amounts of arsenic. The results for Ni suggest that certain elements may be readsorbed to the newly exposed surface during the course of the test. Metal cations removed are greatest for the EP procedure, as might be expected, but substantially lower for arsenic than the IAEA test. This is most likely due to the lower pH of the EP test where arsenic is much less readily released to solution than at the higher pH values of the IAEA test.

In contrast, the largest percentages of iron, chromium, and nickel were extracted from the ammonium oxalate extraction of the dewatered sludges of Indianapolis and Pontiac. After incineration the largest percentages of iron, chromium, and nickel were found in the CDB or crystalline fraction. Table 21 shows that the oxalate to CDB ratio (or amorphous to crystalline oxide fraction) decreases as a result of incineration as bound waters of hydration are driven off. Arsenic remained in the oxalate fraction of the dewatered sludge samples and in the sludge ash samples of both Indianapolis and Pontiac indicating a stable mineral association. The data suggets that chromium, nickel, and arsenic are associated with the iron oxyhydroxides but more specific research would be required to demonstrate this.

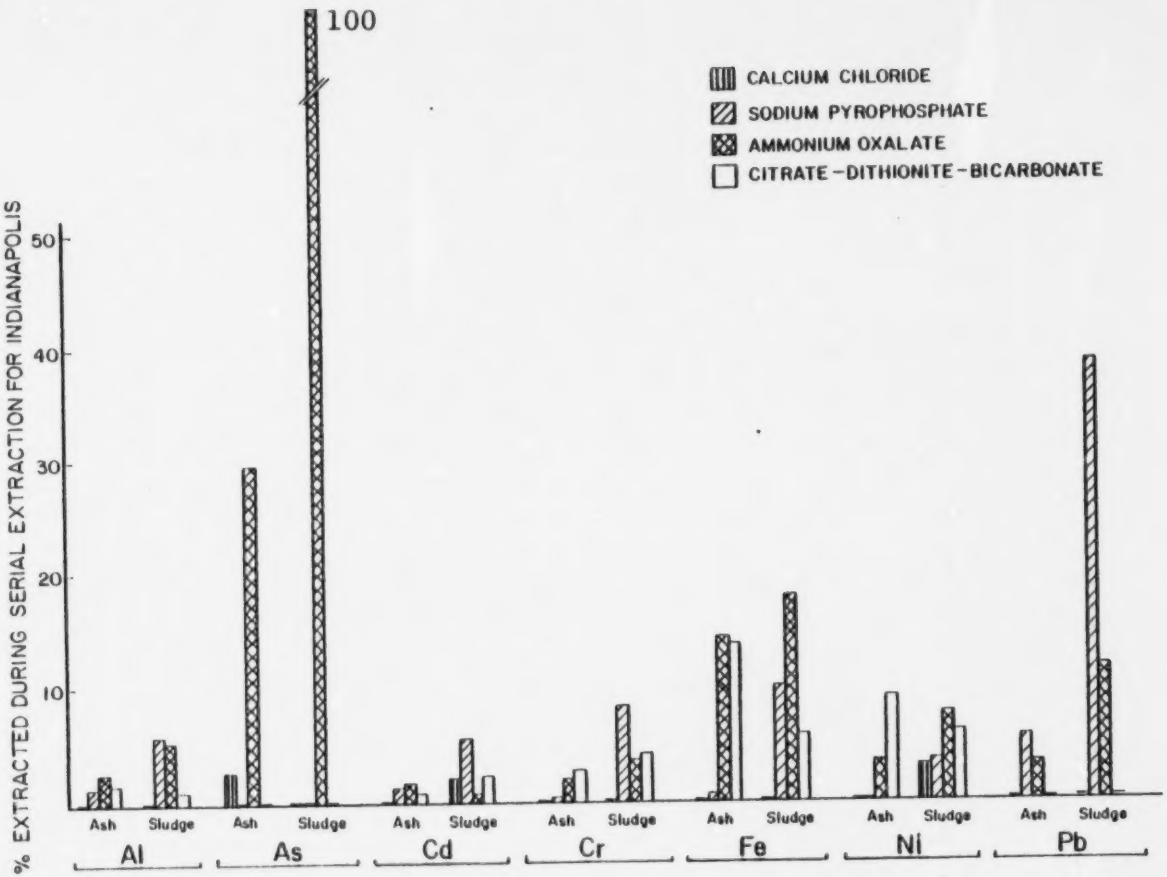

Figure 7. Serial extraction procedure results for Indianapolis.

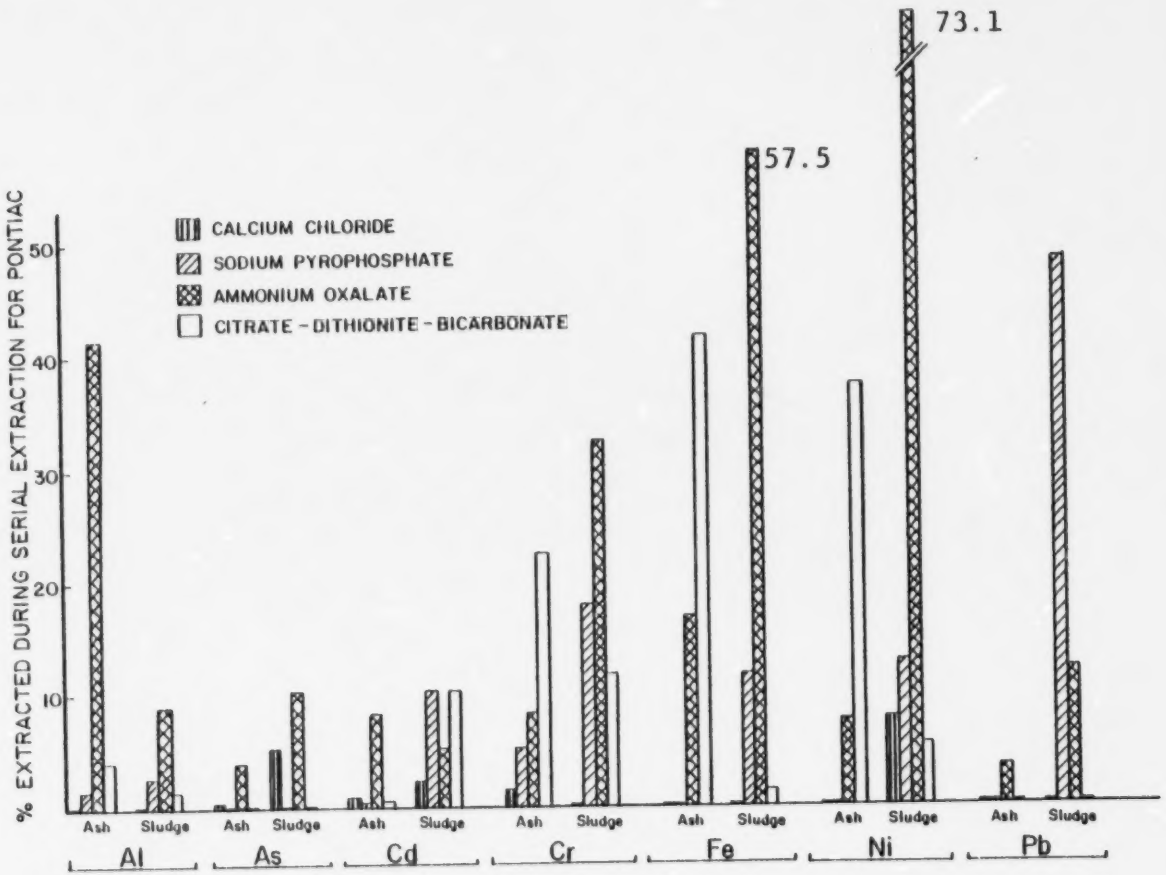

Figure 8. Serial extraction procedure results for Pontiac.

TABLE 20. RELATIVE COMPARISON OF THREE LEACHING TESTS AVERAGE VALUES FOR 10 ASHES

ELEMENT	EP	IAEA	ASTM-A*
As	297	800	1
Cd	239	15	1
Cr	41	5	1
Ni	839	0.5	1
Pb	95	12	1

*ASTM-A NORMALIZED TO UNITY

TABLE 21. OXALATE:CBD RATIO FROM SERIAL EXTRACTION PROCEDURE

LOCATION	ASH						
	Al	As	Cd	Cr	Fe	Ni	Pb
DETROIT	2.3	CDB < D.L.*	3.1	0.6	1.1	1.7	CDB < D.L.
INDEPENDENCE	4.7	CDB < D.L.	60.7	0.7	0.3	0.1	CDB < D.L.
INDIANAPOLIS	1.5	CDB < D.L.	2.2	0.7	1.0	0.4	CDB < D.L.
JERSEY CITY	14.2	CDB < D.L.	140.0	0.4	0.5	0.3	CDB < D.L.
PONTIAC	10.9	CDB < D.L.	40.0	0.4	0.4	0.2	CDB < D.L.
PORT HURON	8.3	CDB < D.L.	50.0	1.3	0.6	0.2	CDB < D.L.

LOCATION	DEWATERED SLUDGE						
	Al	As	Cd	Cr	Fe	Ni	Pb
INDIANAPOLIS	5.9	CDB < D.L.	0.3	0.9	3.1	1.3	CDB < D.L.
PONTIAC	8.0	CDB < D.L.	0.5	2.8	49.9	14.0	CDB < D.L.

*D.L. - Detection Limit

CONCLUDING REMARKS ON LEACHATE AND EXTRACTION DATA

As a result of the single-reagent and serial-extraction tests that were run, a model of sludge ash surfaces in an aqueous environment is suggested. The surface of these ash particles very likely display a mosaic pattern of dominant minerals as depicted in Figure 9. This variation also exists with depth into the particle as shown in Figure 10. These minerals dominate the solution chemistry, controlling such factors as pH and major ionic concentrations. Although discrete trace element minerals or co-precipitates may exist in the ashes, it is suggested that their behavior in an aqueous environment follows a model similar to that shown in Figure 11 where surface bonding to primarily inorganic oxides occurs.

The data suggest further that the addition of Fe(III) and/or Al (III) salts tends to partition metals from organic to inorganic forms in raw sludges. Upon incineration the inorganic fractions of Fe and Al become more crystalline, which acts to increase the leachability of metal cations if the iron and aluminum salts have been specifically added during treatment.

AMES TEST RESULTS

Measurements for spontaneous reversion rates for all strains and positive controls with and without activation (S-9 liver homogenate) were made and found to conform well with results cited in the literature. Only positive results from the Ames testing are presented in the body of the report. All data (positive and negative) are given in Appendix B.

Results of the Ames test were analyzed by two different methods to determine mutagenicity of the ash samples. The first analysis was that suggested by Ames and used by a number of researchers[86,87,88]. By this "rule of thumb", a test compound is considered mutagenic if the number of mutations induced by that compound is twice (or greater) the level of spontaneous revertants for the strain being tested.

The second method was the statistical analyses designed by Katz[89]. By this means, the test sample is defined as mutagenic at a 5% significance level (95% confidence interval) if $\theta > 1.64$.

$$\theta = \frac{a(M+0.5) - b(m+0.5)}{\sqrt{ab(M+m)}}$$

where

- a = number of control plates
- b = number of test plates
- M = total number of test plate revertants
- m = total number of spontaneous revertants from the control plates

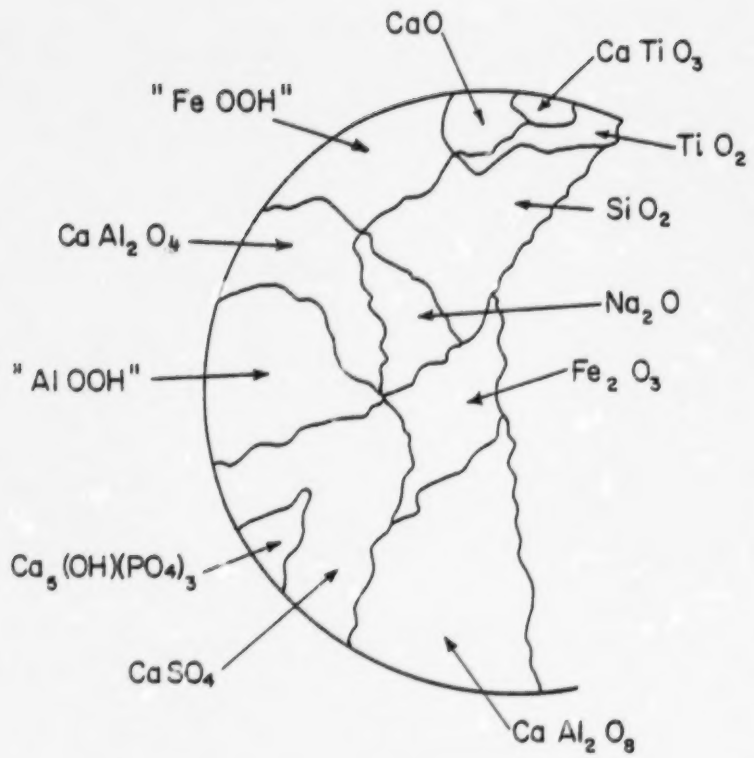

Figure 9. Major minerals of ash.

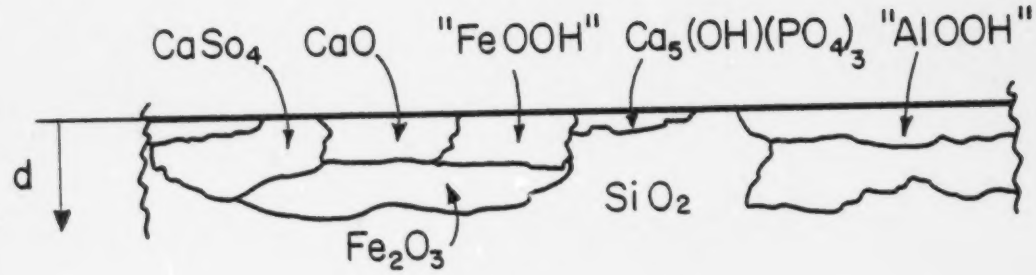

FIGURE 10. Schematic depth profile of ash.

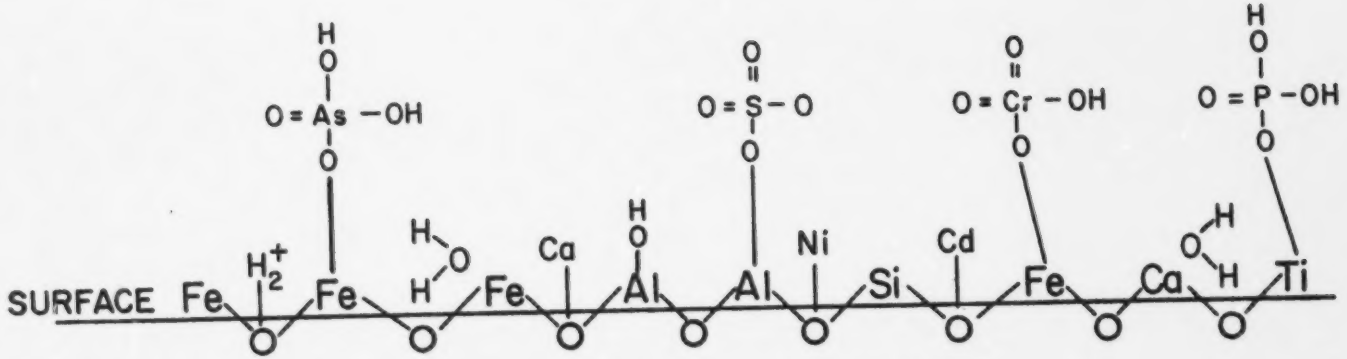

FIGURE 11. Typical surface assocations in solution.

A number of metals concentrated by the incineration process are contained in and can be leached from sewage sludge ash as indicated previously in this section of the report. However, the danger of these metals is not assessed by the Ames test because the metals are unable to enter the Salmonella to damage its genetic structure due to the hindering effect of citrate, phosphate, and Mg salts in the minimal agar. Therefore, the results of this research are representative of organic components of the ash which are present at much lower levels than metals. Far more positives were obtained in tests of extractions designed to collect organics than those of inorganic extractions. This strongly suggests that mutagenic activity was being expressed by organic-extractable material which could include PAH and halogenated hydrocarbons (two classes of compounds whose presence has been determined in ash from municipal incinerators[58,59,61],

The inorganic extractions yielding positive results by the Katz criteria included hydroxylamine hydrochloride, ammonium oxalate, sodium pyrophosphate, and calcium chloride as well as water. This may indicate that mutagenic compounds in the ash are associated with surface oxides being either slightly bound by these oxides or simply adsorbed onto their surface. Aqueous extractions also produced positive results (with both the Ames and Katz analyses) suggesting that mutagens can be leached from landfill ash by the action of rainfall and surface waters alone.

The extraction method resulting in the greatest number of positives by both analysis techniques was the resin collection system for aqueous ash extracts. Three ashes responded with positive induced reversion levels, as did the sludge sample, with this extraction. This indicates that mutagens are present in an aqueous extract, but in concentrations below detection in the Ames assay. Test results from several unconcentrated aqueous extractions also suggested the presence of mutagenic compounds which could be detected without being collected and concentrated.

Negative results in the Ames test indicates that neither base pair nor frameshift mutations occurred at the histidine operon of the tester bacteria. However, this does not discount the possibility that some mutation has occurred. Negative results of any back mutation test such as the Ames assay (as opposed to a forward mutation system) will result even if mutations have actually been effected but are located at an inappropriate site on the gene.

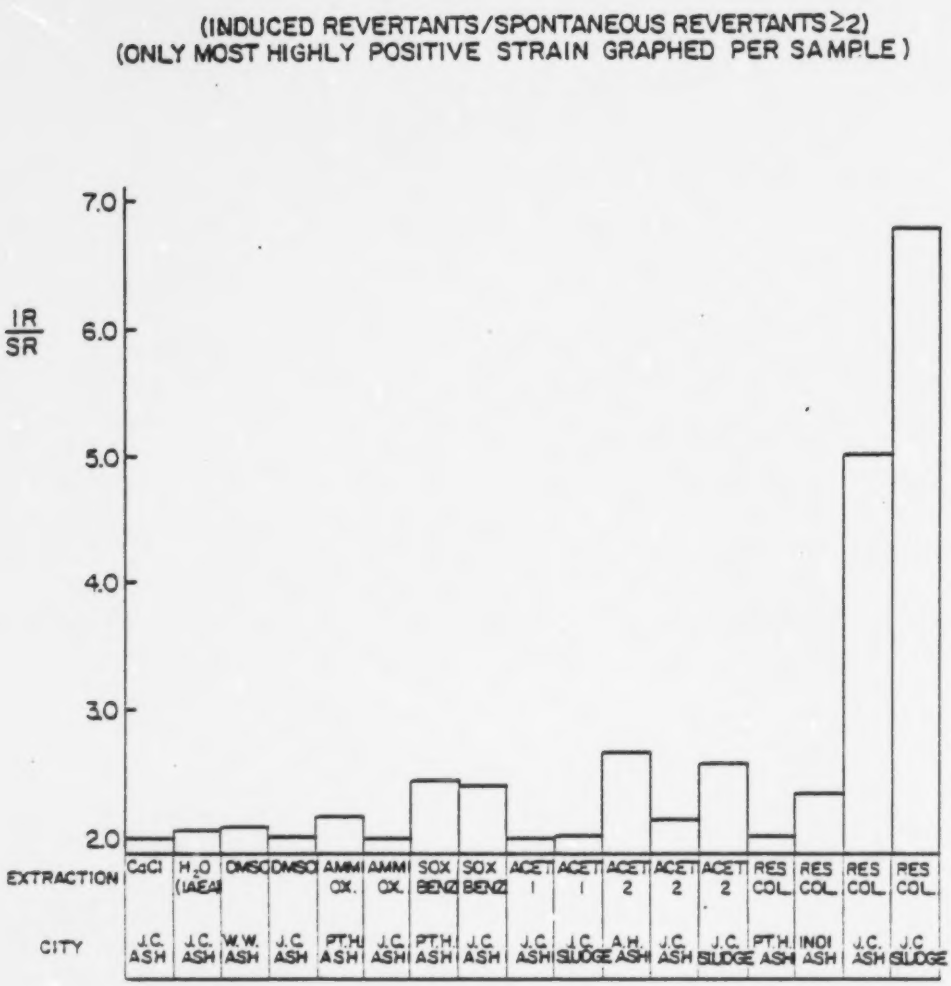

Figure 12. Test positives with Ames Analysis.

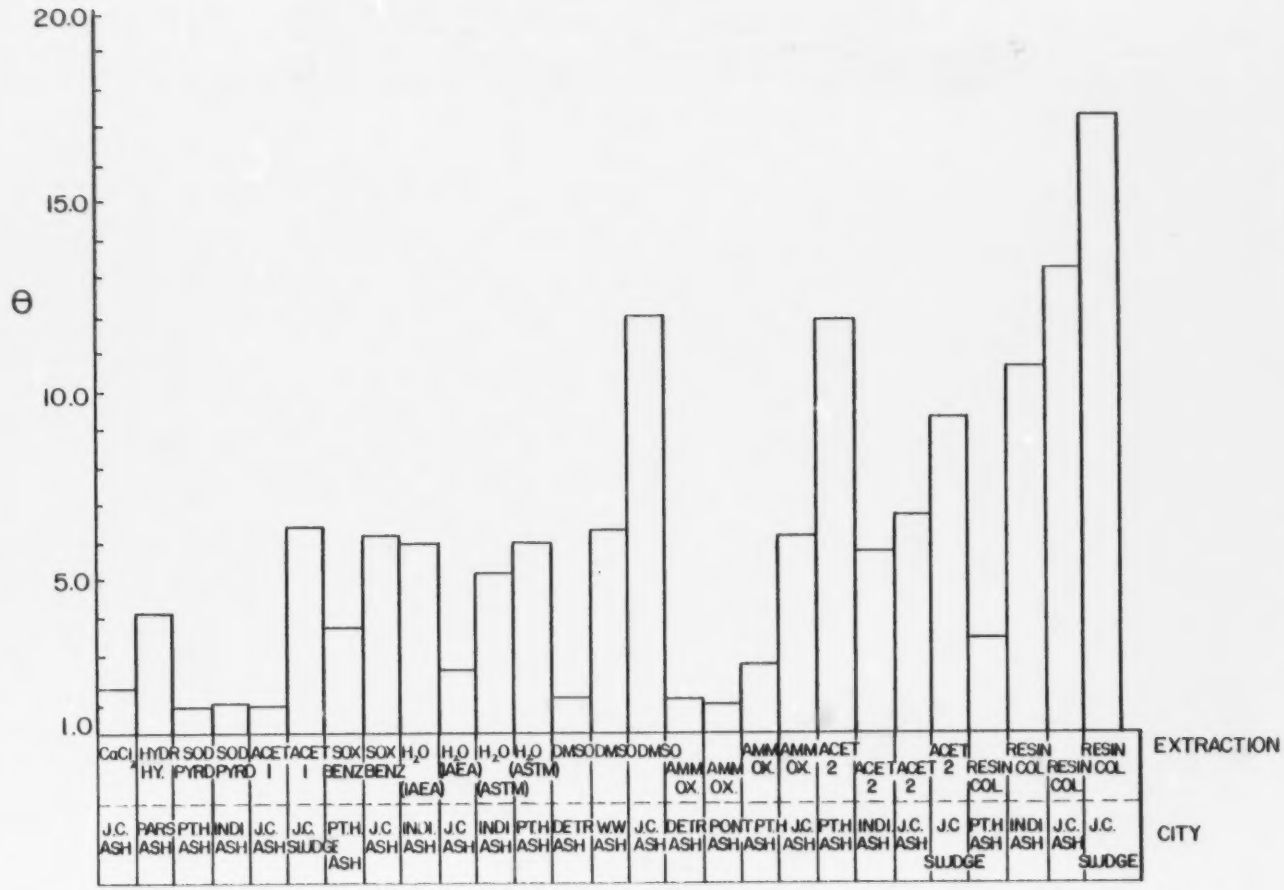

Figure 13. Test positives with Katz analysis (only most highly positive strain graphed per sample)

TABLE 22. POSITIVE RESULTS OF ASH ASSAYS

CITY	EXTRACTION	STRAIN	S-9 (+/-)	PLATE COUNTS (test plates/controls)			TOTALS	IR/SR (Ames)[a]	θ (Katz)[b]
Detroit	DMSO	1538	+	25 20	29 20	30 21	84 61	1.38	1.83
Detroit	Ammonium Oxalate	1537	+	11 6	11 7	12 7	34 20	1.70	1.77
Pontiac	Ammonium Oxalate	1537	+	10 6	11 6	11 7	32 19	1.68	1.68
Parsippany	Hydroxylamine Hydrochloride	100	+	160 138	161 139	164 143	485 420	1.15	2.13
Waldwick	DMSO	98	+	78 37	80 41	82 41	240 119	2.02	6.33
Waldwick	DMSO	1535	+	35 18	39 18	40 19	114 55	2.07	4.46
Port Huron	H_2O (ASTM)	98	-	71 39	74 40	76 40	221 119	1.85	6.02
Port Huron	Ammonium Oxalate	1537	+	13 6	14 7	16 7	43 20	2.15	2.77
Port Huron	Sodium Pyrophosphate	100	+	153 139	155 139	162 143	470 420	1.12	1.64
Port Huron	$Acetone_2$ (50 g ash)	98	+	57 36	63 38	64 39	184 113	1.63	4.06
Port Huron	$Acetone_2$	100	+	274 138	283 138	287 142	844 418	2.02	11.97

a = positive mutagen if induced revertants/spontaneous revertants $\geq$ 2.
b = positive mutagen if θ > 1.64.

TABLE 22 continued

CITY	EXTRACTION	STRAIN	S-9 (+/-)	PLATE COUNTS (test plates/controls)			TOTALS	IR/SR (Ames)[a]	θ (Katz)[b]
Port Huron	$Acetone_2$	1535	+	52 19	55 20	56 22	163 61	2.67	6.75
Port Huron	Soxhlet Benzene	100	+	172 141	180 145	182 146	534 432	1.86	3.25
Port Huron	Soxhlet Benzene	1537	+	18 8	20 8	21 8	59 24	2.46	3.73
Port Huron	Resin Column	100	-	174 139	179 142	181 143	534 424	1.26	3.52
Port Huron	Resin Column	1537	-	13 7	13 7	15 7	43 21	2.05	2.63
Indianapolis	H_2O (IAEA)	98	+	75 37	76 40	79 40	230 117	1.97	6.01
Indianapolis	H_2O (ASTM)	1538	-	47 25	51 26	53 26	151 77	1.96	5.23
Indianapolis	Sodium Pyrophosphate	1537	+	12 7	12 8	12 8	37 23	1.61	1.68
Indianapolis	$Acetone_2$ (50 g ash)	100	+	197 137	200 140	209 142	606 419	1.45	5.81
Indianapolis	$Acetone_2$	1535	+	39 21	41 22	42 22	122 65	1.88	4.10
Indianapolis	$Acetone_2$	1537	-	12 7	12 7	12 8	36 22	1.64	1.71

a = positive mutagen if induced revertants/spontaneous revertants ≥ 2.
b = positive mutagen if $\theta > 1.64$.

TABLE 22 continued

CITY	EXTRACTION	STRAIN	S-9 (+/-)	PLATE COUNTS (test plates/controls)			TOTALS	IR SR (Ames)[a]	$\hat{\theta}$ (Katz)[b]
Indianapolis	Resin Column	100	-	272	275	280	827	1.91	10.66
				142	145	146	433		
Indianapolis	Resin Column	1537	-	18	18	18	54	2.35	3.42
				7	8	8	23		
Jersey City	H_2O (IAEA)	1537	+	14	14	15	43	2.05	2.63
				7	7	7	21		
Jersey City	Ammonium Oxalate	98	-	76	80	82	238	2.00	6.24
				38	39	42	119		
Jersey City	$CaCl_2$	1535	+	35	35	38	108	2.00	4.16
				17	17	20	54		
Jersey City	$CaCl_2$	1537	-	11	11	12	34	1.70	1.77
				6	7	7	20		
Jersey City	DMSO	100	-	279	281	289	849	2.01	11.95
				137	142	143	422		
Jersey City	DMSO	1535	+	36	37	37	110	2.00	4.20
				17	18	20	55		
Jersey City	DMSO	1537	+	11	12	14	37	1.95	2.27
				6	6	7	19		
Jersey City	Soxhlet Benzene	98	+	76	80	81	238	2.00	6.24
				38	39	42	119		
Jersey City	Soxhlet Benzene	100	-	156	160	163	479	1.18	1.86
				139	140	143	422		

a = positive mutagen if induced revertants/spontaneous revertants ≥ 2.
b = positive if $\theta > 1.64$.

TABLE 22 continued

CITY	EXTRACTION	STRAIN	S-9 (+/-)	PLATE COUNTS (test plates/controls)			TOTALS	IR/SR (Ames)[a]	θ (Katz)[b]
Jersey City	Soxhlet Benzene	1537	+	17 8	19 8	19 8	55 24	2.29	3.37
Jersey City	Soxhlet Benzene	1538	+	50 20	52 21	52 23	154 64	2.41	6.03
Jersey City	Acetone$_1$ (25 g ash)	98	+	73 38	78 38	79 39	230 115	2.00	6.14
Jersey City	Acetone$_1$	1538	+	32 24	32 26	36 27	100 77	1.30	1.65
Jersey City	Acetone$_2$ (50 g ash)	98	+	79 38	83 38	85 40	247 116	2.13	6.82
Jersey City	Acetone$_2$	1537	+	12 8	13 8	13 8	38 24	1.52	1.65
Jersey City	Acetone$_2$	1538	+	47 25	48 26	50 26	145 77	1.88	4.50
Jersey City	Resin Column	98	+	80 38	83 40	84 41	247 119	2.08	6.64
Jersey City	Resin Column	100	-	282 139	289 141	290 142	861 422	2.04	12.23
Jersey City	Resin Column	100	+	249 138	251 140	260 143	760 421	1.81	9.84
Jersey City	Resin Column	1537	+	20 7	21 7	21 7	62 21	2.95	4.39
Jersey City	Resin Column	1538	+	107 21	111 22	114 23	332 66	5.03	13.28

a = positive mutagen in induced revertants/spontaneous revertants $\geq$ 2.
b = positive if $\theta > 1.64$.

TABLE 23. POSITIVE RESULTS OF SLUDGE ASSAYS

CITY (SLUDGE)	EXTRACTION	STRAIN	S-9 (+/-)	PLATE COUNTS (test plates/controls)			TOTALS	$\frac{IR}{SR}$ (Ames)[a]	θ (Katz)[b]
Jersey City sludge	Acetone 1 (25 g sludge)	98	+	80	82	85	247	2.01	6.39
				40	40	43	123		
Jersey City sludge	Acetone 2 (50 g sludge)	98	-	84	88	89	261	2.19	7.23
				38	39	42	119		
Jersey City sludge	$Acetone_2$	98	+	104	107	110	322	2.58	9.27
				41	41	43	125		
Jersey City sludge	$Acetone_2$	100	-	219	228	230	677	1.58	7.46
				142	143	143	428		
Jersey City sludge	$Acetone_2$	100	+	196	197	204	597	1.42	5.48
				138	140	143	421		
Jersey City sludge	$Acetone_2$	1535	+	45	46	46	137	2.36	5.58
				18	20	20	58		
Jersey City sludge	$Acetone_2$	1538	-	33	34	34	101	1.42	2.21
				23	24	24	71		
Jersey City sludge	$Acetone_2$	1538	+	50	53	54	157	2.18	5.55
				23	24	25	72		
Jersey City sludge	Resin Column	98	-	89	93	95	277	2.31	7.83
				40	40	40	120		
Jersey City sludge	Resin Column	98	+	102	106	106	314	2.71	9.50
				37	39	40	116		
Jersey City sludge	Resin Column	100	-	288	296	300	884	2.11	12.89
				138	138	142	418		
Jersey City sludge	Resin Column	100	+	199	206	210	615	1.46	5.96
				139	139	144	422		
Jersey City sludge	Resin Column	1535	-	42	43	46	131	2.22	5.15
				20	20	19	59		
Jersey City sludge	Resin Column	1537	+	28	29	29	86	4.30	6.31
				6	7	7	20		
Jersey City sludge	Resin Column	1538	+	155	159	163	477	6.81	17.36
				22	23	25	70		

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APPENDIX A

SERIAL EXTRACTION PROCEDURE

1. Add 50 mℓ of 0.05 M calcium chloride (pH 6.2) to 5 grams of dried sample (10:1 solution to solid ratio). Provide continuous mixing for 16 hours. Transfer contents to a centrifuge bottle and centrifuge at 2500 rpm for 15 minutes. Filter the extract with a 0.45 μm Millipore filter membrane. Place any residue on the filterpad with the original sample. Acidify the extract with 0.2 mℓ nitric acid and dilute to 100 mℓ with double distilled water.

2a. Add 400 mℓ of 0.1 M sodium pyrophosphate (pH 10.0) to the material from step 1 (80:1 solution to solid ratio). Provide continuous mixing for 16 hours. Transfer contents to a centrifuge bottle and centrifuge at 2500 rpm for 15 minutes. Filter the extract with a 0.45 μm membrane. Place any residue on the filterpad with the solid sample.

b. Wash the sample from step 2a. with 30 mℓ of 0.1 M sodium chloride. Centrifuge and filter as described above. Combine extracts from a and b. Acidify the extract with 0.9 mℓ of nitric acid and dilute to 450 mℓ with double distilled water.

3a. Add 65 mℓ of double strength acid/oxalate reagent (0.2 M oxalic acid/0.4 M ammonium oxalate), pH 3.3, to the 5 grams of material from step 2. Provide mixing for 1 hour. Centrifuge and filter as described above. Place the extract in a clean sample bottle.

b. Wash the material from 3a. with 75 mℓ of single strength acid/oxalate reagent (0.1 M oxalic acid/0.2 M ammonium oxalate). Centrifuge and filter. Combine the extract from 3b. with 3a.

c. Add 125 mℓ of single strengh acid/oxalate to the material from 3b. Provide mixing for 1 hour. Centrifuge and filter. Combine the extract with a and b.

d. Wash the material with 30 mℓ of 0.1 M sodium chloride. Centrifuge and filter. Combine extracts from a, b, and c and acidify with 0.7 mℓ of nitric acid. Dilute to 350 mℓ with double distilled water.

4a. Add 500 mℓ of 0.22 M sodium citrate/0.11 M sodium bicarbonate solution, pH 5.6, to the solid sample. Immerse in a water bath maintained at 85°C for 15 minutes.

b. Add 10 grams of sodium dithionite (iron free) and maintain at 85°C for a further 15 minutes, stirring at intervals.

c. Allow mixture to cool. Centrifuge at 2500 rpm for 15 minutes. Filter the extract and replace any residue on the filter pad. Place the extract in a clean sample bottle.

d. Wash the material from 4c. with 300 mℓ of 1 M sodium chloride. Centrifuge and filter. Transfer the extract to the sample bottle.

e. Allow the sample bottle to stand exposed to the atmosphere for 2-3 days until the development of a yellowish-brown color indicates that the oxidation of dithionite is complete.

f. Acidify the extract with 1.6 mℓ of nitric acid and dilute to 800 mℓ with double distilled water (due to evaporation).

APPENDIX B

AMES TEST RESULTS

For each category reported (i.e., extraction/strain tested), the data are given as two sets of numbers. These are the raw colony counts. The top row gives the number of induced revertant colonies and the bottom row gives the number of spontaneous revertant colonies. Normally, tests for each extraction/strain were made in triplicate.

TABLE B.1. Independence Ash

EXTRACTION \ STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538
H_2O (ASTM)	40 41 43[a]	140 141 145	20 20 20	7 8 9	24 25 24
	39 39 40[b]	140 142 143	19 20 20	7 7 7	22 24 24
H_2O (IAEA)	41 41 43	140 143 145	20 21 21	7 8 8	20 23 25
	39 39 40	140 142 143	19 20 20	7 7 7	22 24 24
HNO_3	42 43	139 142	20 20	7 7	25 25
	40 40 42	137 140 142	19 21 22	7 7 8	23 24 25
HYDROXYLAMINE HCl	42 42	139 141	21 22	9 9	25 26
	39 40 41	137 138 143	20 20 20	6 8 8	24 24 26
$CaCl_2$	41 41	140 143	20 22	8 9	26 27 28
	39 39 40	140 142 143	19 20 20	7 7 7	24 24 25
AMMONIUM OXALATE	43 43	144 146	20 23	9 9	26 27
	39 40 41	137 138 143	20 20 20	6 8 8	24 24 26
SODIUM PYROPHOSPHATE	43 45	141 144	22 23	9 10	26 28
	40 41 41	139 140 144	20 21 21	7 8 8	22 23 25
NaOH	40 41	140 143	20 21	7 7	24 24
	40 40 42	137 140 142	19 21 22	7 7 8	23 24 25
ACETIC ACID	43 43	143 145	22 22	8 8	25 26
	40 40 42	137 140 142	19 21 22	7 7 8	23 24 25
DMSO (1 day)	42 44 45	144 144 146	20 22 24	8 9 9	25 27 28
	39 39 40	140 142 143	19 20 20	7 7 7	22 24 24
DMSO (1 wk)	41 42 45	141 145 148	21 22 24	9 9 9	26 28 29
	38 40 40	143 143 146	17 20 21	7 7 9	25 25 25
DMSO (1 wk) + S9	42 45 46	140 143 148	23 24 25	9 10 10	26 26 28
	38 39 40	136 140 142	21 21 22	7 7 7	24 25 25

[a] Induced revertants [b] spontaneous revertants

TABLE B-1 continued

STRAIN / EXTRACTION	TA 98			TA 100			TA 1535			TA 1537			TA 1538		
ACETONE 1	41	43	44	145	145	149	22	24	25	8	8	9	26	27	28
	40	40	40	137	144	144	20	20	23	6	7	8	23	24	24
ACETONE 2	41	44	44	143	147	148	20	23	23	9	9	11	28	29	30
	38	40	41	138	140	143	18	20	21	6	8	8	24	24	25
ACETONE 2 + S9	43	45	46	146	147	151	24	24	24	9	10	11	27	30	30
	39	40	40	140	140	145	19	21	21	7	7	7	23	25	26
RESIN COLUMN	47	48	48	144	148	149	22	24	25	10	10	10	28	31	32
	38	40	41	138	140	143	18	20	21	6	8	8	24	24	25
RESIN COLUMN + S9	45	48	50	150	151	152	23	25	26	10	11	12	29	29	29
	39	40	40	140	140	145	19	21	21	7	7	7	23	25	26

TABLE B-2. Montrose Ash

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
H_2O (ASTM	41 41 42 39 39 40	143 144 144 140 142 143	20 20 21 19 20 20	7 8 8 7 7 7	24 24 24 22 24 24
H_2O (IAEA)	40 41 42 39 39 40	140 144 145 140 142 143	20 21 21 19 20 20	8 8 8 7 7 7	23 24 25 22 24 24
HNO_3	40 40 37 38 40	139 142 138 143 143	20 20 18 20 21	7 8 6 8 8	22 24 23 25 25
HYDROXYLAMINE HCl	42 43 39 41 42	140 144 139 140 144	19 22 20 20 20	8 8 7 7 8	24 25 24 24 24
$CaCl_2$	41 44 39 39 40	144 147 140 142 143	21 22 19 20 20	8 8 7 7 7	23 26 22 24 24
AMMONIUM OXALATE	43 45 39 41 42	140 145 140 142 145	22 24 19 21 22	8 10 7 7 9	25 25 24 25 25
SODIUM PYROPHOSPHATE	42 43 40 41 41	141 145 139 140 144	23 23 20 21 21	9 9 7 8 8	24 26 22 23 25
NaOH	40 40 37 38 40	140 144 138 143 143	20 20 18 20 21	7 7 6 8 8	24 24 23 25 25
ACETIC ACID	40 43 37 38 40	142 147 138 143 143	19 21 18 20 21	9 9 6 8 8	21 25 23 25 25
DMSO (1 day)	41 42 43 40 40 42	142 146 148 140 143 144	20 21 22 20 20 20	8 8 8 7 7 8	25 27 27 24 24 24
DMSO (1 wk)	43 43 44 39 40 42	144 147 150 139 143 144	21 23 25 20 21 21	8 9 9 7 7 7	26 26 27 23 24 25
DMSO (1 wk) + S9	43 45 46 40 40 41	143 148 149 140 141 145	24 24 24 19 20 21	8 10 10 6 7 8	26 27 30 25 25 26
DMSO (1 wk) ASH INCLUDED	44 44 45 39 40 42	140 148 148 139 143 144	20 24 24 20 20 20	8 9 10 7 7 7	25 26 28 23 24 25

TABLE B-3. Green Bay Ash

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
H_2O (ASTM)	41 41 42 38 40 40	138 142 145 141 141 144	21 21 21 18 20 20	7 7 7 7 7 8	24 25 25 24 24 24
H_2O (IAEA)	40 40 40 38 40 40	140 141 146 141 141 144	20 21 21 18 20 20	7 8 8 7 7 8	21 25 25 24 24 24
HNO_3	40 42 40 41 41	136 142 137 140 142	20 20 20 20 21	7 7 7 7 7	22 25 23 25 25
HYDROXYLAMINE HCl	41 41 39 40 42	137 142 139 140 144	20 22 19 21 21	7 8 6 7 8	25 27 22 24 25
$CaCl_2$	39 42 38 40 40	140 146 138 143 145	21 21 20 20 20	7 7 7 7 8	26 27 24 25 25
AMMONIUM OXALATE	39 43 40 41 41	140 143 137 140 142	22 23 20 20 21	7 8 7 7 7	26 28 23 25 25
SODIUM PYROPHOSPHATE	40 43 39 40 42	138 144 139 140 144	20 20 19 20 21	7 8 6 7 8	26 26 22 24 25
NaOH	40 40 40 41 41	137 141 137 140 142	19 21 20 20 21	6 7 7 7 7	24 25 23 25 25
ACETIC ACID	42 43 40 41 41	140 145 127 140 142	21 23 20 20 21	7 7 7 7 7	25 26 23 25 25
DMSO (1 day)	41 42 43 38 40 40	140 147 148 138 143 145	22 22 23 20 20 20	8 8 8 7 7 8	25 27 28 24 25 25
DMSO (1 wk)	44 44 44 39 40 42	146 149 150 139 143 144	23 23 25 20 21 21	8 8 9 7 7 7	26 28 29 23 24 25
DMSO (1 wk) + S9	42 42 43 38 39 40	144 148 151 136 140 142	24 24 26 21 21 22	9 9 9 7 7 7	27 27 27 24 25 25
DMSO (1 wk) ASH INCLUDED	43 44 44 39 40 42	147 148 152 139 143 144	23 25 25 20 21 21	8 9 9 7 7 7	27 28 28 23 24 25

TABLE B-4. Pontiac Ash

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
H_2O (ASTM)	41 42 44 40 40 40	141 144 145 139 143 143	22 22 22 19 20 21	8 8 8 7 7 8	25 25 25 22 23 25
H_2O (IAEA)	40 41 43 40 40 40	140 142 145 139 143 143	20 22 23 19 20 21	7 7 8 7 7 8	23 24 25 22 23 25
HNO_3	40 40 39 39 41	140 144 137 141 143	20 21 19 21 21	8 9 6 7 7	25 25 24 24 24
HYDROXYLAMINE HCl	42 43 40 40 40	144 147 143 143 146	22 24 20 20 20	10 10 7 7 9	25 27 23 24 25
$CaCl_2$	43 44 38 40 40	143 147 140 140 146	23 24 20 22 22	8 8 7 7 7	23 26 20 24 24
AMMONIUM OXALATE	44 44 40 41 41	145 150 138 140 144	20 23 19 21 22	11 13 7 7 9	27 27 24 24 26
SODIUM PYROPHOSPHATE	42 43 38 40 40	144 148 140 140 146	23 25 20 22 22	8 8 7 7 7	23 25 20 24 24
NaOH	40 42 39 39 41	140 143 137 141 143	20 24 19 21 21	7 8 6 7 7	21 25 24 24 24
ACETIC ACID	43 45 39 39 41	144 144 137 141 143	21 24 19 21 21	8 9 6 7 7	25 27 24 24 24
DMSO (1 day)	40 43 43 39 39 40	144 147 151 140 142 143	22 23 19 20 20	8 9 7 7 7	25 27 27 22 24 24
DMSO (1 wk)	42 42 44 38 40 40	146 147 153 143 144 146	23 23 17 20 21	9 9 7 7 9	26 28 29 25 25 25
DMSO (1 wk) + S9	42 43 44 38 38 40	143 143 148 136 140 142	25 25 21 21 22	8 9 7 7 7	26 26 28 24 25 25
RETEST OF AMMONIUM OXALATE				11 12 12 7 7 8	
AMMONIUM OXALATE + S9 [c]	42 42 45 40 40 41	144 146 149 138 141 144	24 25 20 21 22	11 12 12 7 7 7	25 26 28 23 24 24

[c]Retest data are presented in Table 22 of report

TABLE B-5. Parsippany Ash

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
H_2O (ASTM)	41 41 43 39 39 39	143 146 146 140 142 145	20 21 21 19 19 19	7 8 8 7 7 8	25 25 25 25 25 26
H_2O (IAEA)	40 40 43 39 39 39	142 142 146 140 142 145	20 20 23 19 19 19	7 8 8 7 7 8	25 26 26 25 25 25
HNO_3	40 42 40 41 44	142 144 139 140 145	20 21 19 21 22	7 7 7 7 7	25 27 24 25 25
HYDROXYLAMINE HCl	43 44 40 40 40	158 162 143 143 146	26 27 20 20 20	7 8 7 7 9	26 26 23 24 25
$CaCl_2$	42 44 37 39 40	141 144 135 141 142	20 23 20 20 21	6 8 7 7 7	27 27 23 25 26
AMMONIUM OXALATE	43 43 40 41 41	146 147 138 140 144	22 25 19 21 22	8 8 7 7 9	26 27 24 24 26
SODIUM PYROPHOSPHATE	38 42 37 39 40	144 145 135 141 142	22 23 20 20 21	8 8 7 7 7	25 27 23 25 26
NaOH	42 42 40 41 44	141 144 139 140 145	20 21 19 21 22	7 7 7 7 7	25 25 24 24 26
ACETIC ACID	44 46 40 41 44	145 148 139 140 145	24 24 19 21 22	8 9 7 7 7	25 26 24 24 26
DMSO (1 day)	43 46 48 39 42 42	144 147 149 141 141 145	23 23 25 20 20 20	7 7 8 6 6 7	26 27 29 25 25 25
DMSO (1 wk)	40 44 48 40 43 44	142 145 147 137 139 139	24 26 26 19 22 22	8 8 8 7 7 8	27 27 27 24 24 25
DMSO (1 wk) + S9	43 45 46 40 40 42	143 144 148 140 141 145	23 26 26 20 20 22	8 8 10 7 7 7	27 28 29 23 25 25
RETEST OF [C] HYDROXYLAMINE HYDROCHLORIDE		153 157 158 138 142 144	25 27 28 21 21 21		
HYDROXYLAMINE HYDROCHLORIDE + S9	42 42 42 40 40 42	156 158 163 140 141 145	26 26 27 20 20 22	8 8 9 7 7 7	25 26 26 23 23 25

[C]Retest data are presented in Table 22 of report.

TABLE B-6. Detroit Ash

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
H_2O (ASTM)	40 42 44 40 40 40	144 144 146 139 143 143	20 21 21 19 20 21	8 8 8 7 7 8	23 24 24 22 23 25
H_2O (IAEA)	41 41 43 40 40 40	140 142 145 139 143 143	20 20 20 19 20 21	7 8 8 7 7 8	21 25 25 22 23 25
HNO_3	40 41 39 39 41	138 142 137 141 143	20 22 19 21 21	7 7 6 7 7	25 25 24 24 24
HYDROXYLAMINE HCl	42 43 40 40 40	141 145 143 143 146	23 23 20 20 20	7 8 7 7 9	22 26 23 24 25
$CaCl_2$	41 41 38 40 40	143 147 140 140 146	23 24 20 22 22	6 8 7 7 7	25 27 20 24 24
AMMONIUM OXALATE	43 44 40 41 41	145 149 138 140 144	24 24 19 21 22	11 12 7 7 9	26 26 24 24 26
SODIUM PYROPHOSPHATE	42 43 38 40 40	141 145 140 140 146	23 24 20 22 22	8 8 7 7 7	25 26 20 24 24
NaOH	41 41 39 39 41	144 146 137 141 143	20 22 19 21 21	7 7 6 7 7	25 25 24 24 24
ACETIC ACID	40 42 39 39 41	141 145 137 141 143	23 23 19 21 21	8 9 6 7 7	26 27 24 24 24
DMSO (1 day)	42 43 45 39 39 40	143 146 147 140 142 143	22 24 26 19 20 20	7 7 8 7 7 7	24 25 25 22 24 24
DMSO (1 wk)	44 45 45 38 40 40	145 145 149 143 143 146	23 23 23 17 20 21	7 8 8 7 7 9	29 30 31 25 25 25
DMSO (1 wk) + S9	46 46 48 38 39 40	130 143 147 136 140 142	24 25 25 21 22 22	8 8 8 7 7 7	32 34 34 24 25 25
RETEST OF [c] DMSO (1 wk) + S9	46 48 49 40 40 41				30 32 33 23 24 24
RETEST OF AMMONIUM OXALATE				10 12 12 7 7 8	
AMMONIUM OXALATE + S9 [c]	42 44 44 40 40 41			11 11 13 7 7 7	25 26 26 23 24 24

[c]Retest data are presented in Table 22 of report.

TABLE B-7. Waldwick Ash

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
H_2O (ASTM)	40 41 42 38 40 40	143 143 146 141 141 144	20 21 21 18 20 20	7 7 7 7 7 8	25 25 25 24 24 24
H_2O (IAEA)	40 40 41 38 40 40	140 144 144 141 141 144	19 19 21 18 20 20	7 7 8 7 7 8	24 24 25 24 24 24
HNO_3	40 40 39 39 41	140 145 137 141 143	20 20 19 21 21	7 7 6 7 7	23 24 24 24 24
HYDROXALIMINE HCl	38 42 39 40 42	141 144 139 140 144	24 24 19 21 21	7 8 6 7 8	25 25 22 24 25
$CaCl_2$	41 44 40 41 41	143 147 137 140 142	23 24 20 20 21	7 8 7 7 7	24 25 23 25 25
AMMONIUM OXALATE	43 44 40 41 41	142 142 138 140 144	20 23 19 21 22	8 8 7 7 9	22 26 24 24 26
SODIUM PYROPHOSPHATE	42 44 40 41 41	141 146 139 140 144	24 24 20 21 21	9 9 7 8 8	23 25 22 23 25
NaOH	40 40 39 39 41	140 141 137 141 143	20 21 19 21 21	6 7 6 7 7	24 24 24 24 24
ACETIC ACID	41 43 39 39 41	144 148 137 141 143	23 25 19 21 21	7 8 6 7 7	26 27 24 24 24
DMSO (1 day)	42 47 49 38 40 40	143 148 150 138 143 145	25 25 29 20 20 20	8 8 8 7 7 8	25 26 28 24 25 25
DMSO (1 wk)	47 49 49 39 40 42	147 151 154 139 143 144	24 28 29 20 21 21	8 8 9 7 7 7	26 26 29 23 24 25
DMSO (1 wk) + S9	68 71 72 38 39 40	146 153 153 136 140 142	42 44 45 21 21 22	8 9 9 7 7 7	27 28 29 24 25 25
RETEST OF DMSO (1 wk) + S9	45 49 50 39 39 41	148 154 155 139 139 144	25 26 27 20 20 20		
RETEST OF DMSO (1 wk) C + S9	67 73 74 40 40 41	149 149 155 138 141 144	40 43 44 20 21 22	8 9 10 7 7 7	25 28 28 23 24 24

[C]Retest data are presented in Table 22 of report

TABLE B-8. Indianapolis Ash

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
H_2O (ASTM)	43 43 44 39 39 39	144 147 148 140 142 145	23 23 24 19 19 19	8 8 9 7 7 8	46 49 50 25 25 26
H_2O (IAEA)	47 47 48 39 39 39	143 148 148 140 142 145	21 22 24 19 19 19	8 8 8 7 7 8	30 31 34 25 25 26
HNO_3	42 43 40 41 44	145 147 139 140 145	23 23 19 21 22	6 7 7 7 7	25 25 24 25 25
HYDROXYLAMINE HCl	45 45 40 40 40	148 149 143 143 146	24 25 20 20 20	8 8 7 7 9	26 27 23 24 25
$CaCl_2$	44 45 37 39 40	144 148 135 141 142	23 24 20 20 21	8 9 7 7 7	27 27 23 25 26
AMMONIUM OXALATE	43 46 40 41 41	141 147 138 140 144	24 24 19 21 22	9 9 7 7 9	24 27 24 24 26
SODIUM PYROPHOSPHATE	44 46 37 39 40	144 148 135 141 142	23 25 20 20 21	11 11 7 7 7	28 28 23 25 26
NaOH	42 44 40 41 44	145 145 139 140 145	20 22 19 21 22	7 8 7 7 7	25 26 24 24 26
ACETIC ACID	45 48 40 41 44	143 148 139 140 145	24 26 19 21 22	8 9 7 7 7	28 28 24 24 26
DMSO (1 day)	45 45 46 39 42 42	148 148 153 141 141 145	22 24 25 20 20 20	8 8 8 6 6 7	26 28 29 25 25 25
DMSO (1 wk)	46 48 49 40 43 44	145 150 152 137 139 139	23 23 26 19 22 22	8 9 9 7 7 8	28 28 29 24 24 25

TABLE B-8 continued

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
DMSO (1 wk) + S9	45 49 49 40 40 42	147 152 155 140 141 145	24 24 24 20 20 22	9 9 9 7 7 7	27 29 30 23 25 25
ACETONE 1	45 46 49 40 40 40	150 153 158 137 144 144	24 25 25 20 20 23	9 9 10 6 7 8	26 27 29 23 24 24
ACETONE 2 [C]	46 46 50 38 40 41	152 152 157 138 140 143	35 39 40 18 20 21	12 12 12 6 8 8	27 27 31 24 24 25
ACETONE + S9 [C]	47 50 50 40 40 42	153 153 160 137 141 144	26 26 27 20 20 20	11 11 12 7 7 7	25 29 29 22 25 26
RESIN COLUMN [C]	45 48 50 38 40 41	151 153 161 138 140 143	26 26 26 18 20 21	17 17 18 6 8 8	26 28 31 24 24 25
RESIN COLUMN + S9	46 49 50 39 40 40	154 157 162 140 140 145	25 26 28 19 21 21	10 12 12 7 7 7	28 30 33 25 25 26
RETEST 1 OF H_2O (ASTM) [C]					44 48 49 24 25 25
H_2O (ASTM) +S9	42 45 45 40 40 40	144 148 149 140 141 145	24 24 24 20 20 22	8 9 9 7 7 7	30 30 31 23 25 25
RETEST 1 OF SODIUM PYROPHOSPHATE				11 11 12 7 7 8	
SODIUM PYROPHOSPHATE + S9 [C]	43 45 46 38 42 42	145 146 150 140 140 147	24 26 26 19 19 19	12 13 13 7 7 7	28 29 30 25 25 26
RETEST 1 OF H_2O (IAEA)	48 48 49 37 41 41				
H_2O (IAEA) +S9 [C]	70 73 76 38 40 40	145 147 151 140 140 146	23 23 25 20 21 22	8 8 8 7 7 7	30 30 31 24 24 25

[C] Retest data are presented in Table 22 of report

TABLE B-8. Port Huron Ash

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
H_2O (ASTM)	70 70 75 39 39 40	148 151 152 140 142 143	22 22 23 19 20 20	8 8 8 7 7 7	25 25 26 22 24 24
H_2O (IAEA)	47 48 50 39 39 40	144 149 151 140 142 143	20 22 22 19 20 20	7 8 8 7 7 7	24 25 26 22 24 24
HNO_3	41 41 37 38 40	140 145 138 143 143	21 21 18 20 21	7 7 6 8 8	24 24 23 25 25
HYDROXYLAMINE HCl	43 44 38 40 41	143 147 136 140 142	24 25 20 20 21	7 8 7 7 7	26 27 24 25 25
$CaCl_2$	42 44 39 39 40	141 146 140 142 143	21 23 19 20 20	8 8 7 7 7	23 25 22 24 24
AMMONIUM OXALATE	45 45 39 41 42	148 149 140 142 145	24 24 19 21 22	12 12 7 7 9	27 27 24 25 25
SODIUM PYROPHOSPHATE	43 45 38 40 41	152 158 136 140 142	22 23 20 20 21	8 9 7 7 7	25 26 24 25 25
NaOH	40 42 37 38 40	145 148 138 143 143	20 21 18 20 21	7 7 6 8 8	24 25 23 25 25
ACETIC ACID	41 41 37 38 40	146 150 138 143 143	23 23 18 20 21	8 9 6 8 8	26 26 23 25 25
DMSO (1 day)	43 43 46 40 40 42	142 147 147 140 143 144	24 24 25 20 20 20	8 8 8 7 7 8	25 27 27 24 24 24
DMSO (1 wk)	41 44 44 39 40 42	147 150 151 139 143 144	25 25 25 20 21 21	8 9 9 7 7 7	26 26 26 23 24 25

TABLE B-9 continued

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
DMSO (1 wk)	45 45 45	150 153 153	23 26 26	7 9 9	28 29 29
+ S9	40 40 41	140 141 145	19 20 21	6 7 8	25 25 26
SOXHLET BENZENE	46 46 48	149 154 158	24 26 29	9 9 10	29 30 32
	40 40 40	137 141 144	20 20 20	7 7 7	24 26 26
SOXHLET [C] BENZENE + S9	48 49 50	153 160 163	27 27 27	10 12 12	30 30 30
	40 41 43	140 141 146	20 21 21	7 7 7	23 24 25
ACETONE 1	43 43 43	147 149 154	24 26 26	8 10 10	26 27 27
	40 40 40	137 144 144	20 20 23	6 7 8	23 24 24
ACETONE 1 + S9	43 44 45	152 154 157	23 25 27	9 9 10	27 27 27
	39 40 40	140 140 145	19 21 21	7 7 7	23 25 26
ACETONE 2	47 47 51	150 153 156	24 27 27	9 10 11	28 28 30
	38 40 41	138 140 143	18 20 21	6 8 8	24 24 25
ACETONE 2 + S9 [C]	60 61 65	269 277 280	49 51 52	10 10 10	27 27 29
	40 40 42	137 141 144	20 20 20	7 7 7	22 25 26
RESIN COLUMN [C]	47 48 48	170 171 177	26 26 26	13 15 15	30 31 32
	38 40 41	138 140 143	18 20 21	6 8 8	24 24 25
RESIN COLUMN	46 48 50	153 154 161	26 27 27	10 12 12	32 32 33
+ S9	39 40 40	140 140 145	19 21 21	7 7 7	25 25 26
RETEST 1 OF [C] H_2O (ASTM)	72 73 75				
	37 41 41				
H_2O (ASTM) + S9	47 49 50	150 150 154	24 24 24	7 8 8	26 26 28
	40 40 40	140 141 145	20 20 22	7 7 7	23 25 25
RETEST 1 OF AMMONIUM OXALATE				11 11 13	
				7 7 8	
AMMONIUM OXALATE [C]	44 46 46	143 145 149	25 25 25	14 14 15	27 29 29
+ S9	40 40 41	138 141 144	20 21 22	7 7 7	23 24 24
RETEST 1 OF SODIUM PYROPHOSPHATE		153 157 162			
		139 143 146			
SODIUM PYROPHOSPHATE [C]	45 45 47	155 160 163	22 23 23	8 8 10	27 28 29
+ S9	38 42 42	140 140 147	19 19 19	7 7 7	25 25 26
SOXHLET ETHANOL	48 48 48	154 156 161	26 27 27	9 9 9	29 30 31
	39 39 40	141 143 146	20 20 21	7 7 7	24 25 25
SOXHLET ETHANOL	47 50 50	155 159 162	25 25 28	11 11 11	30 30 31
+ S9	40 41 42	140 142 145	19 20 20	7 7 8	24 24 24

[C]Retest data are presented in Table 22 of report

TABLE B-10. Jersey City Ash

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
H_2O (ASTM)	43 43 44 39 39 39	147 151 154 140 142 145	21 22 22 19 19 19	10 10 10 7 7 8	26 28 29 25 25 26
H_2O (IAEA)	42 44 44 39 39 39	144 152 153 140 142 145	21 21 21 19 19 19	11 11 13 7 7 8	27 27 27 25 25 25
HNO_3	42 43 40 41 44	147 150 139 140 145	20 21 19 21 22	7 7 7 7 7	25 25 24 25 25
HYDROXYLAMINE HCl	43 43 40 40 40	145 149 143 143 146	22 22 20 20 20	7 8 7 7 9	26 27 23 24 25
$CaCl_2$-1 [C]	41 44 37 39 40	140 144 135 141 142	23 24 20 20 21	12 12 7 7 7	26 26 23 25 26
AMMONIUM OXALATE	73 77 39 41 42	141 143 140 142 145	22 23 19 21 22	11 11 7 7 9	27 28 24 25 25
SODIUM PYROPHOSPHATE	43 45 37 39 40	140 145 135 141 142	22 24 20 20 21	9 9 7 7 7	26 26 23 25 26
NaOH	44 44 40 41 44	145 149 139 140 145	21 21 19 21 22	7 8 7 7 7	25 27 24 24 26
ACETIC ACID	46 47 40 41 44	147 152 139 140 145	24 25 19 21 22	8 10 7 7 7	27 27 24 24 26
DMSO (1 day)	43 44 44 39 42 42	151 153 160 141 141 145	24 24 25 20 20 20	9 9 9 6 6 7	27 27 29 25 25 25
DMSO (1 wk) [C]	46 46 46 40 43 44	273 279 289 137 139 139	27 27 27 19 22 22	10 10 11 7 7 8	26 28 29 24 24 25

[C]Retest data are presented in Table 22 of report

TABLE B-10 continued

EXTRACTION \ STRAIN	TA 98	TA 100	TA 1535	TA 1537	TA 1538
DMSO (1 wk) [c]	45 47 48	153 153 164	25 26 27	10 11 11	30 30 30
+ S9	40 40 42	140 141 145	20 20 22	7 7 7	23 25 25
ACETONE 1	48 48 49	149 150 153	24 25 25	9 9 10	27 29 29
	40 40 40	137 144 144	20 20 23	6 7 8	23 24 24
ACETONE 1 [c]	75 78 81	148 152 153	23 23 24	10 10 10	31 32 32
+ S9	37 40 40	136 141 142	19 20 20	7 7 7	24 24 24
ACETONE 2	47 47 50	149 149 154	24 24 24	10 12 12	30 30 33
	38 40 41	138 140 143	18 20 21	6 8 8	24 24 25
ACETONE 2 [c]	73 80 82	150 153 158	23 25 25	11 12 12	46 46 49
+ S9	40 40 42	137 141 144	20 20 20	7 7 7	22 25 26
RESIN COLUMN [c]	47 50 50	272 283 286	26 26 26	10 12 12	31 31 33
	38 40 41	138 140 143	18 20 21	6 8 8	24 24 25
RESIN COLUMN [c]	80 80 85	258 261 267	25 27 28	19 21 21	122 123 128
+ S9	39 40 40	140 140 145	19 21 21	7 7 7	25 25 26
SOXHLET [c]	46 50 51	155 161 164	26 26 27	11 11 12	32 33 33
BENZENE	40 40 40	137 141 144	20 20 20	7 7 8	24 26 26
SOXHLET [c]	77 80 82	153 158 161	25 28 28	14 15 15	53 53 54
BENZENE	40 41 43	140 141 146	20 21 21	7 7 7	23 24 25
+ S9					
RETEST 1 OF				10 11 11	
H_2O (IAEA)				6 7 7	
H_2O (IAEA) + S9 [c]	46 48 48	142 144 147	21 23 23	14 14 14	26 26 27
	40 40 40	140 141 145	20 20 22	7 7 7	23 25 25
$CaCl_2$-1 [c]			36 39 39	11 12 12	
+ S9[2]			19 19 20	7 7 7	
SOXHLET	46 47 47	153 153 162	26 26 26	10 11 11	30 31 32
ETHANOL	39 39 40	141 143 146	20 20 21	7 7 7	24 25 25
SOXHLET	49 49 49	153 157 160	25 25 26	11 11 12	30 30 31
ETHANOL	40 41 42	140 142 145	19 20 20	7 7 8	24 24 24
+ S9					
RETEST 1 OF [c]	72 72 78				
AMMONIUM	40 41 43				
OXALATE					
AMMONIUM	48 49 51	145 147 150	24 27 27	9 9 9	28 28 29
OXALATE	40 40 41	138 141 144	20 21 22	7 7 7	23 24 24
+ S9					

[c]Retest data are presented in Table 22 of report

TABLE B-11. Jersey City Sludge

STRAIN / EXTRACTION	TA 98	TA 100	TA 1535	TA 1537	TA 1538
ACETONE 1	48 49 49	155 157 160	26 26 26	8 8 9	29 29 30
C	40 40 40	137 144 144	20 20 23	6 7 8	23 24 24
ACETONE 1 + S9	77 80 82	149 149 155	25 27 27	8 9 9	27 30 30
C	37 40 40	136 141 142	19 20 20	7 7 7	24 24 24
ACETONE 2	81 85 88	210 213 222	25 26 28	11 11 11	34 34 35
C	38 40 41	138 140 143	18 20 21	6 8 8	24 24 25
ACETONE 2 + S9	91 9[illegible] 96	192 201 204	46 46 46	10 11 12	52 53 53
C	40 40 42	137 141 144	20 20 20	7 7 7	22 25 26
RESIN COLUMN	85 91 93	283 284 293	44 44 45	10 12 12	31 31 33
C	38 40 41	138 140 143	18 20 21	6 8 8	24 24 25
RESIN COLUMN + S9	102 104 107	194 199 207	27 27 27	29 29 30	170 171 175
C	39 40 40	140 140 145	19 21 21	7 7 7	25 25 26

[c]Retest data are presented in Table 22 of report